Lab on a Chip

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Organ-on-a-chip devices advance to market

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To curb the high cost of drug development, there is an urgent need to develop more predictive tissue models using human cells to determine drug efficacy and safety in advance of clinical testing. Recent insights gained through fundamental biological studies have validated the importance of dynamic cell environments and cellular communication to the expression of high fidelity organ function. Building on this knowledge, emerging organ-on-a-chip technology is poised to fill the gaps in drug screening by offering predictive human tissue models with methods of sophisticated tissue assembly. Organ-on-a-chip start-ups have begun to spawn from academic research to fill this commercial space and are attracting investment to transform the drug discovery industry. This review traces the history, examines the scientific foundation and envisages the prospect of these renowned organ-on-a-chip technologies. It serves as a guide for new members of this dynamic field to navigate the existing scientific and market space.

Introduction

The increasing expense of drug development is a strong contributor to today's skyrocketing healthcare costs. Spending on drug development has increased over the past 20 years while the number of drugs approved annually has declined.[1,2] Today it takes nearly 2.5 billion dollars and 10–12 years on average to develop one clinically applicable drug.[3] Two thirds of the total drug development costs are spent in the clinical trial stage.[4] To curb the cost of drug development, it is important to improve the predictive power of pre-clinical screening to eliminate ineffective drug candidates as early as possible. In fact, there is an increasing emphasis on the notion of "fail early and fail cheaply" in the big pharmaceutical companies. In addition, many compounds with tremendous potential health benefit are eliminated early in development due to

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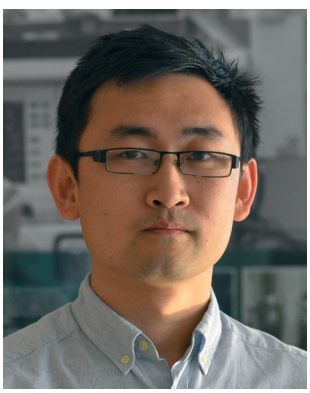

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the lack of predictability in the pre-clinical models. Other industries that regularly develop new chemicals for consumer use, such as cosmetic, agro-food and consumer goods, face similar challenges.

The ever-increasing speed and resolution of genomic sequencing, and comprehensive cataloging of species' germline DNA over the past three decades, has facilitated homologous sequence-function association of organisms of complexity ranging from worms to humans. With this map of conserved genes in hand, major therapeutic targets were identified, and non-human species were exploited in drug development relatively quickly. Yet, although this shows promise for drug investigation, proteome and interactome[5] studies of the last decade have revealed that small inter-species sequence differences at the genome level cascade through transcriptional, translational, and post-translational levels leading to major aggregate "physiological" differences in drug specificity, activity and toxicity between species.

Hence, the traditional drug developmental models of single cell screening and animal testing often fail to predict drug effects observed at the human clinical trial stage.[4] These late stage failures significantly drive up the costs and patient risk. In fact, it is estimated that less than 8% of successful animal trials for cancer drugs translate to successful human clinical trials,[6] primarily due to the differences in the physiology of different species. Drug candidates entering clinical trials are often shown to be ineffective (*e.g.*, antineoplastic drug Targretin, repurposed to treat Alzheimer's, exhibited equivocal efficacy in humans[7]), to have high risk of side effects (*e.g.*, anti-arrhythmic drugs encainide and flecainide presenting higher proarrhythmia and morbidity in cardiac ischemia, infarction and congestive heart failure patients[8,9]), or even to be fatal (hepatitis B drug, fialuridine, leading to death[10]). Moreover, even drugs that enter the market could still fail and result in catastrophic losses. Cardiac and liver toxicities are the main causes of post-approval drug withdrawals, while many other drugs fail due to the lack of efficacy and poor understanding of the mechanism of action. Tegaserod, sibutramine, propoxyphene, Vioxx and rosiglitazone[11] have been withdrawn from the market due to severe cardiovascular toxicity. Vioxx developed by Merck has been linked to over 27 000 deaths between 1999 and 2003 and resulted in more than $5 billion in criminal and civil settlements.[12]

There is a clear need for improvement of the predictive power of preclinical studies through more accurate modeling of human physiology.[4,13] To meet this need, organ-on-a-chip technology[14–17] was hatched through the convergence of two areas of research: microfluidics and tissue engineering (Fig. 1). On the basis of microfluidic fabrication techniques,[15] a foundation was formed to build complex physical environments emulating the normal organ environment of the cells. Tissue engineering techniques[18] used to make large tissue implants have also been scaled down to make micro-tissues. Advances in the field of stem cell biology[19,20] further accelerated the progress in this field by providing easy access to non-malignant human cell lines and patient-specific stem cells, making this technology more relevant to human and even patient specific physiology. The emergence of organ-on-a-chip technology was rooted in the recognition that traditional cell culture in 2D cannot adequately recapitulate all aspects of tissue level function, such as modeling the tissue-to-tissue interface and simulating the dynamics of living organs.[14,21–23] These high-level tissue functions modeled with human cells are the key to accurately predicting drug responses in humans.[14,21,24,25]

Achieving organs of relevant physiological function is no easy task. Bioengineers look to optimize assembly of single cells in specific spatial arrangements and train them coordinately to express their native organ-level physiological function.[26] Unlike animal testing, in these miniaturized tissue models we can directly access cells within their tissue architecture to probe their functional change under drug stimulation in real time. These devices have tremendous potential to provide us with high value data to illuminate subtle drug effects and to allow for direct examination of a drug's mechanism of action. The emergence of organ-on-a-chip technology is built on decades of fundamental biological studies in cell morphogenesis, cell–cell interaction, and biomechanics,[27–32] recognizing that the environment is as important as the cells. In fact, each time a new environmental factor is considered, such as topographical guidance,[33] mechanical stimulation,[34–36] biochemical gradient,[37,38] and spatially defined co-culture,[39–42] an additional function emerges.

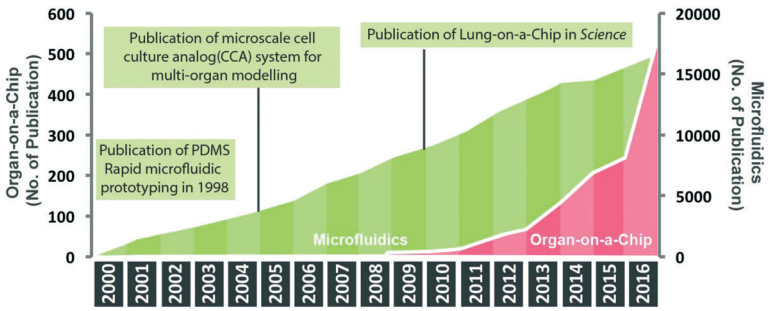

Fig. 1 Rise in academic publishing in organ-on-a-chip space. The key word "Microfluidics" or "Organ-on-a-Chip" was searched on Google scholar. The number of publications found from the search for each corresponding year was plotted from 2000 to 2015. The rise in publication exemplifies the increasing activity and interests in the respective field.

While organ-on-a-chip technologies have yet to substantially integrate into the existing drug development pipeline, a number of organ-on-a-chip start-ups have been established to productize the lab-scale prototypes and to occupy this market space with their own unique ways to perform tissue assembly.[26] The market potential of this technology sector is large, but the bar is also set very high. As a baseline, these organ-on-a-chip companies would need to replicate the extensive historical ADME-TOX (absorption, distribution, metabolism and excretion/toxicity) database of some thoroughly studied and characterized compounds. Further efforts are ongoing to compare their predictive validity against traditional cell culture and to demonstrate that the high value data generated is worth the additional cost. In addition, validation studies are critically needed to clearly benchmark the functional properties of the organ-on-a-chip devices against the organ function in an adult human to prove physiological relevance. While most research papers provide a set of characterizations, adoption of these systems for drug testing requires strong quantitative proof that the native tissue function is truly reproduced in terms of action potential recordings, force development, enzyme function, permeability, gene expression, protein translation, metabolic function, and system response to a challenge, among others. Gene expression analysis can be very useful to directly compare various *in vitro* and *in vivo* models and to uncover differences in detection of transcriptional changes under drug perturbation. Studies that adopted this type of analysis already revealed important differences in *in vitro* and *in vivo* models across different species.[43]

This review will trace the history and progress of several prominent organ-on-a-chip technologies from the bench to the commercialization stage, which is currently performed mostly through the start-up companies. We will also review the scientific foundation these businesses are built on, and provide recommendation for future studies that will help to solidify the impact of organ-on-a-chip technologies leading to the transformative effects on the drug development process. We have focused our analysis on commercialized technologies as they tend to be more extensively developed and closer to making an impact in the pharmaceutical industry. Since this is a dynamic field, new commercial entities emerge rapidly and the availability of public data inadvertently varies vastly in the commercial sector. Herein, we analyzed each technology solely based on the available scientific publication and press reports. Therefore, it is important to note that this review is not meant to be comprehensive, but rather specifically tailored as a navigation tool for newcomers to this dynamic field in the existing scientific and commercial space.

In this review, we define organ-on-a-chip as technologies that seek to improve cellular functions by creating high-level tissue function *in vitro* with advanced microfabrication techniques to better predict the effects of drugs or other compounds in the human body. Moreover, we broadly grouped these existing commercial entities into three categories (body-on-a-chip, tissue interface-on-a-chip, parenchymal tissue-on-a-chip) based on the overall technology (Fig. 2). The categorization utilizes the distinct technical emphasis on (1) the modeling of systematic interaction between various tissues and organs of the body (body-on-a-chip), (2) the barrier function within each organ (tissue interface-on-a-chip), and (3) the functionality of the parenchymal tissue (parenchymal tissue-on-a-chip). However, this does not imply that these three aspects of human physiology are not interactive. In fact, they are inter-dependent. Organ-on-a-chip technology seeks to model high-level tissue function by including additional environmental factors, but they are also often designed to model specific aspects of human physiology, hence understanding the omissions and assumptions in each model is just as critical. It is important to note that this categorization was based on the initial focus of these companies, but their technical innovation in tissue assembly is often versatile enough to allow for expansion to potentially cover all three aspects in the future. Although the commercialized platform from each company does not necessarily represent the most advanced technology in their respective scientific community, they do occupy the commercial space through their own unique technological advantages. Finally, the commercial success of a technology not only depends on the degree of innovation, but also its adoptability. The review will also discuss various factors, such as the material selection, tissue scaling, cell sources and format of operation, involved in technology development and adoption.

Body-on-a-chip

Informed by our current knowledge in pharmacokinetics (dynamic concentration profile of drugs) and pharmacodynamics (functional effects of drugs), integrated systems with multiple microscale cellular environments can be designed to simulate the human body and make new predictions about the pharmacokinetics of new drugs. Essentially, pharmacokinetics (PK) models the human body mathematically while body-on-a-chip devices aim to do so physically. However, mimicking the true physiological complexity of how the human body absorbs, distributes, metabolizes, and eliminates (known as ADME) drugs across multiple organs is much more complex than simply connecting various cellular environments. The key to achieving this is proportional scaling of each organ model to reflect the actual physiological relationships between them. Common scaling methods are allometric scaling,[44,45] functional scaling,[46] multi-functional scaling[47] and methods based on organ volume and blood flow residence time.[48] Each scaling method has a different rationale and focus. The multi-dimensionality of the human body makes scaling an extremely complex task. For instance, organ mass does not directly translate to organ functional activity, such that the functional activity of the kidney and lung relies on the tissue surface rather than the tissue mass. More extensive discussions on these scaling methods can be found in other reviews.[49–53]

Dr. Michael Shuler at Cornell University pioneered this field by introducing one of the first body-on-a-chip

Fig. 2 Summary of organ-on-a-chip start-ups and their core products. * indicates the product is still under development. All images used in this figure were reproduced with permission.[40,58,65,66,68,77,79,109,114,134,143,152,155,209,210,226,231,254,267,270,271,273,306–309]

devices,[54–56] dated as early as 2004,[54,55] with a focus on scaling organ models to match physiological liquid-to-cell ratios, organ volumes, and blood residence times. Combining this effort with the expertise of Dr. James Hickman, a pioneer of neuronal culture on microelectrode arrays, Hesperos, Inc was created to offer multi-organ systems with built-in biological sensors (mechanical and chemical) for bioanalytics and systemic toxicology. The key technology of Hesperos, Inc is a pumpless four-organ (heart, liver, neuron, skeletal muscle) system[57] in which the assessment of the toxicological and functional responses of five drugs was demonstrated.[57] The incorporation of built-in sensors offers improved ability for functional readouts from cells, such as contraction frequency for cardiomyocytes, and electrophysiological recording for neurons. The long-term recirculation of a common serum-free medium between the multiple organ compartments is another key innovation that differentiates Hesperos, Inc from the other companies. The elimination of serum in culture media is necessary to reduce the variability in culture conditions which could lead to variable drug testing results. However it is also important to note that there are important transporter proteins present in serum. Removal of serum could be followed by replacing certain carrier proteins that are important to the delivery of hydrophobic molecules to the appropriate organs. One example is vitamin D binding protein, which would be critical to studies of vitamin D transport and metabolism.

The continuous recirculation of media in the system over an extended period (up to two weeks) at physiological cell-to-liquid ratios allows for the accumulation of secreted

biological factors in the common media to physiological levels over time. The achievement of correct scaling and control of fluid residence time of multiple organs with continuous recirculation of fluid is complex, and requires accurate adjustment of flow resistance in the system. This is an important feature that serves as the technological foundation for capturing the pharmacokinetics and pharmacodynamics in the human body. Furthermore, their system adopted gravity driven flow with open-well access to achieve media recirculation without the use of external pumps. This design significantly simplified the setup and improved potential compatibility with prevalent traditional analytical tools including pipettes and plate readers. But, it is also important to recognize that even with long-term re-circulation, there will either have to be removal of toxins and metabolites by a liver and kidney model, or the continuous replacement of medium. The human body replaces about 10% of its free fluid each day, despite having highly effective kidneys and liver. Devices developed by Dr. Shuler and colleagues were made of polydimethylsiloxane (PDMS) at the prototyping stage. PDMS is a common material for microfluidic device fabrication, but is known to absorb hydrophobic drugs. This material could be replaced with tissue culture plastics, such as polystyrene, styrene–ethylene/butylene–styrene copolymers, PMMA, *etc.*, in further device productization.

To demonstrate organ-level interaction, Dr. Shuler's earlier work with a three-organ system[58] (using colon cancer cells (HCT-116), hepatoma cells (HepG2/C3A), and myeloblasts (Kasumi-1)), demonstrated how the drug (tegafur), which itself is non-toxic to the tumor, is first metabolized by liver cells into the active metabolite 5-fluorouracil that exhibits tumor cytotoxicity. Individual cell types are encapsulated into hydrogel disks to carry out their organ-level metabolic functions separately, but when interconnected they approximate the whole body response of an organism, demonstrating the importance of multi-organ interaction in modeling drug efficacy and toxicity that cannot be captured by testing drugs in a single organ. In fact, this type of multi-organ interaction has been demonstrated repeatedly in Dr. Shuler's work.[55,59–62] In an ambitious effort from his lab, Dr. Shuler and colleagues introduced a pumpless 14 compartment microphysiological system in 2016.[48] This system was designed to mimic organ-level physiological interaction by capturing the relationship between the organ volume and the residence time of 13 organs categorized as barrier tissues (skin, GI tract, and lung) and non-barrier tissues (fat, kidney, heart, adrenal glands, liver, spleen, pancreas, bone marrow, brain, muscle).

Another major innovation in the multi-organ platforms came from the work of Dr. Uwe Marx and colleagues from the Institute of Biotechnology at the Technische Universität Berlin. Their platforms were commercialized through a start-up company, TissUse, founded in Germany in 2010 with an initial focus on two- and four-organ models. Instead of using gravity driven flow, the platform contains a built-in micropump driven by an external pneumatic controller.[63] TissUse's devices are primarily constructed of thermoplastics and the presence of PDMS has been minimized to a thin elastic membrane as part of the built-in pneumatic pumps. The platform was designed so that the tissue chamber is open and separated from the closed fluid channels by a membrane. This open configuration allows various tissues to be assembled and prepared externally and then placed into the chamber following device assembly, giving compatibility with clinically relevant tissue biopsies (*e.g.* skin and hair).[64] Furthermore, other sophisticated tissue assembly methods could also be used in combination with this device. For instance, a hanging drop plate[65] has been used to assemble tissue spheroids, which are then supplied to these devices.[66] Their system also incorporated endothelial cells, coating the fluid channel luminal surfaces to form a vascular interface between the fluid and the parenchymal tissue. At approximately 5 dyne cm^{-2}, coated endothelial cells elongated and self-aligned along the flow direction.

In a co-culture of liver microtissues and skin biopsies,[64] the viability and metabolic activity of these tissues were maintained and tracked for up to 28 days. Liver tissue responded predictably to the hepatotoxic anti-diabetic drug troglitazone which was withdrawn in 2000 by the FDA. In another study[66] on the co-culture of liver- and neuro-spheroids, the experiment was performed with single organ device control. The study claims that the duo-organ culture renders the system more sensitive to neurotoxic 2,5-hexanedione when compared to a single-organ system. In this system, the liver micro-tissue was assembled with a hanging drop plate by combining a human hepatocyte cell line (HepaRG) and a hepatic stellate cell line (HHSteC) while the neurospheroids were directly differentiated as 3-D aggregates. The ability to couple other tissue assembly methods with this device was well demonstrated in this case.

In a more complex version of this device, four organs (intestine, liver, skin, and kidney) were co-cultured for up to 28 days.[67] Impressively, this system contains two separate built-in pumps: one for driving the perfusion between the four organ chambers mimicking blood circulation and another for facilitating the drainage of fluids secreted through the kidney epithelial layer to mimic urination. Due to the presence of two types of epithelial cells (kidney proximal tubule cells and intestinal epithelial cells), the system has three distinct fluid compartments: an intestine lumen, a surrogate blood circuit and an excretory circuit. Each fluid compartment was able to maintain a unique glucose concentration, with the highest observed in the blood circuit and the lowest in the excretory circuit.

Over 28 days, intestine, liver and kidney cells showed stable expression of 12 key genes involved in intestinal adsorption, renal reabsorption, secretion and barrier integrity, hepatic function and metabolism. Gene expression of CYP3A4, involved in phase I metabolism of liver, reduced significantly in the first four days and then stabilized, revealing a four-day adaption phase before reaching systemic homeostasis. However, it is difficult to gauge if the level of gene expression

shown is close to that of the normal tissue level. Furthermore, the issue of tissue volume scaling has not been considered in the multi-organ model, which should be addressed to replicate the correct physiological PK–PD. The platform does provide continuous recirculating media, but the cell-to-liquid ratio and organ volume scaling also requires further examination. Nonetheless, the presence of functional activity in all four organs within a single system that contains three distinct fluid compartments to emulate how nutrients are absorbed and secreted by the body is an important milestone in multi-organ models.

CN Bio Innovations, built from the work of Dr. Linda G. Griffith at MIT, also utilizes a perfusion based multi-well plate system.[68–70] To make liver tissues, hepatocytes can be grown in a 3D environment supported by a membrane with perfusion of culture medium driven by built-in micro-pumps. The platform resembles the Transwell plates, but has fluid circulation passing through the membrane. The open plate configuration makes it easy to change media and seed cells with pipettes. The platform is PDMS-free and embedded with built-in micropumps. The simple setup makes it easy to incorporate multiple organs together. The PhysioMimix™ platform, which arose from a collaborative effort between MIT and the Defense Advanced Research Projects Agency (DARPA) Microphysiological Systems Program, aims to integrate up to 10 organs. For multi-organ integration, the group developed a multi-functional scaling approach to adjust design parameters that best satisfy a prescribed objective according to the known *in vivo* pharmacological outcome of a set of drugs.[47,71] The resulting design parameters will then guide the scaling of the physical devices.

A nonprofit entity in Cambridge, Mass., Charles Stark Draper Laboratory is also actively involved in this area and recently announced a partnership with Pfizer to develop a microphysiological system mimicking the gastrointestinal, liver, and vascular systems.[72] Joseph Charest, head of Draper's organ initiative, claimed Draper's PREDICT96 platform will be based on multi-well plate format incorporated with sensors for high-throughput screening.[73] In a recent collaboration with Dr. Teresa Woodruff at Northwestern University, Draper's system (referred to as EVATAR) was shown to be able to simulate the hormone profile of the female 28-day menstrual cycle by linking organ modules representing the ovary, fallopian tube, uterus, cervix and liver.[74] Ovaries and follicles were obtained from a mouse while the other tissues were obtained from a human. Impressively, the change in hormonal profile to that of a pregnancy-like condition was also demonstrated. Furthermore, the reconfigurable setup allows different tissues to be integrated differently, which is a powerful way to elucidate the fundamental mechanism of reproductive function.

In recent years, sophisticated devices have emerged rapidly with the incorporation of four or more organs to offer surprising insights into the mechanisms of action of drugs that cannot be predicted by single-organ models. For multi-organ integration, there might be an optimal level of complexity where we could derive high-value interpretable data on a device that is also sufficiently complex to be robustly reproduced and productized. Perhaps instead of using one general body-on-a-chip device to model everything, multiple specific multi-organ models might be more valuable in answering very targeted clinical questions. For general drug toxicity, a hepato-cardio model might be sufficient in capturing dangerous drugs since nearly 70% of all drug withdrawals from the market are a result of either heart or liver failure. In addition, we expect that in future studies cell lines will be fully replaced with either primary human cells or tissue-specific cells differentiated from human induced pluripotent stem cells to enhance fidelity and enable precision medicine.

Multi-organ interaction is also just one aspect of high-level physiological interaction that organ-on-a-chip technology aims to emulate. Within each organ, organ-on-a-chip technology is also very powerful in simulating the interaction between different sub-types of cells in a spatially defined manner complemented with tissue-specific biomechanical cues. In fact, before we can faithfully model multi-organ interactions, it is important to capture the physiological hallmarks of each individual organ. As we dive deep into each individual organ, we will see a level of complexity on the cellular level that can easily rival the complexity of multi-organ interaction. Below we will examine companies that are focusing their efforts on optimizing individual organs and validating their physiological hallmarks prior to multi-organ integration.

Tissue interface-on-a-chip

Physiological tissues are not constructed of a homogeneous mixture of cells. The specific spatial arrangement of specialized cells within an organ dictates function. Important physiological events and biological complexity often arise at the interface of different specialized cells. This is what Emulate, Inc., founded by Dr. Donald Ingber at Wyss Institute, aims to mimic with the support of a DARPA grant and multiple series of funding from private investors.[75,76] Their device, which is no bigger than a thumb drive, incorporates a polymer membrane sandwiched between two microfluidic channels. On the two sides of the membrane, which is 10 μm thick and patterned with 10 μm microholes, two different types of cells can be cultured to model their cellular interface. The polymer membrane is elastic and can undergo cyclic stretch with a pneumatic controller to mimic the expansion of the alveolar–capillary interface in the lung or the peristaltic motions of the intestinal lumen in the gut, *etc.* Closed micro-channels on either side of the membrane also provide controlled perfusion to mimic various tissue specific fluid flow conditions. To this point the device has successfully modeled various tissue interfaces: lung- (human pulmonary microvascular endothelial cells/alveolar epithelial cells), gut- (human Caco-2 intestinal epithelial cells), kidney- (human proximal tubular epithelial cells), small airway- (human lung airway epithelial

cells/pulmonary microvascular endothelial cells), and bone marrow- (rat bone marrow) on-a-chip.

In the lung-on-a-chip device,[77] which mimics the interface of endothelial cells and alveolar epithelial cells, pulmonary inflammation and infection were emulated with epithelial stimulation by TNF-α which up-regulated I-CAM1 expression in the endothelium. Neutrophils attached to the activated endothelium and subsequently transmigrated to the epithelial layer. In the case of bacterial infection on the epithelial layer, phagocytosis of the bacteria by the transmigrated neutrophils was demonstrated. This series of biological events involving the interplay of four cell types was conveniently visualized in real time under the microscope, thus showcasing the powerful utility of an organ-on-a-chip system in dissecting biological mechanisms.

The device was also used to model drug induced pulmonary edema[78] by perfusing interleukin-2 (IL-2) through the endothelium layer to demonstrate clinically relevant epithelial barrier leakage. Cyclic stretching exacerbated the leakage by three-fold. Again, this process was visualized by the presence of liquid in the lung epithelial chamber and quantified by changes in the membrane permeability. The presence of angiopoietin-1 inhibited the leakage and stabilized the endothelial junctions. A new pharmacological agent, GSK2193874, was tested and demonstrated a similar stabilization effect, exemplifying the predictive capability of the system. This study also demonstrated that pulmonary leakage induced by IL-2 does not require the presence of immune cells, in contrast to previous studies that show the essential participation of lymphocytes and neutrophils in the induction of pulmonary leakage. This example illuminates the power of organ-on-a-chip technology in dissecting biological mechanisms with not only the inclusion of biological factors but also their absence.

Alveolix, founded by Dr. Olivier Guenat, is also commercializing a lung-on-a-chip device for modeling the alveolar barrier.[79] The PDMS-based device contains a similar stretchable porous membrane. However, the stretching was achieved with an external electro-pneumatic controller by bulging the porous membrane like a diaphragm, thus establishing a 3D cyclic strain in all directions. The membrane is exposed in an open-well format allowing cells to be seeded with simple pipetting like in a standard multi-well plate. However, vascular perfusion is missing in this platform. Because of this simplification, multiple devices can be patterned on a single glass slide and controlled by a single pneumatic circuit.

The intestinal lumen is another organ interface that involves fluid flow and mechanical movement. The intestinal interface has been modeled with Caco-2 cells cultured on decellularized porcine jejunal segments sandwiched in a small-scale bioreactor,[80] and improved cell morphology and expression were shown under dynamic perfusion. Emulate's gut-on-a-chip device[81] further miniaturized this setup and has shown that the presence of low shear flow and cyclic stretch can induce a columnar epithelium with polarized human Caco-2 intestinal epithelial cells on one side of the membrane. This morphology was also previously simulated artificially with microfabricated pillars[82,83] or by explanted crysts[83,84] but was not captured with the conventional static Transwell® systems. Although Caco-2 is a commonly available cell source, future studies could benefit from a more clinically relevant primary cell source or stem cells. Long-term co-culture of bacteria (*Lactobacillus rhamnosus* GG) with the intestine epithelium was not previously possible in static Transwell® systems due to rapid bacterial overgrowth. Emulate's microfluidic based system, which functions as a continuous flow bioreactor, constantly clears the non-adherent bacteria and allows for stable bacterial culture. Interestingly, the presence of bacteria improved epithelial integrity, illuminating the importance of probiotic commensal bacteria in the human gut.[85] The technology was more recently expanded to model virus infection and replication in the gut chips and demonstrated directional secretion of infectious virions and inflammatory cytokines from the cell apex.[86]

In many organ interfaces, the fluid flow is the most significant biomechanical cue, while cyclic stretching is not required. In these cases, the device was simplified with a non-stretchable polyester membrane. In Emulate's kidney-on-a-chip device,[87] the presence of fluid shear alone was sufficient to improve the function of kidney proximal tubular cells cultured alone on one side of the membrane as demonstrated through: restoration of columnar morphology; increase in localized Na^+/K^+ ATPase expression; and improved uptake of albumin, glucose, *etc.* The proximal tubular epithelium also appeared more resistant to drug (cisplatin) injury and exhibited faster recovery.

Similarly, in Emulate's small airway-on-a-chip device,[88] human primary airway epithelial cells were co-cultured against the membrane with lung microvascular endothelial cells. This device demonstrated a robust, polarized epithelial barrier, the presence of cilia, mucociliary transport, and active synchronized cilia beating, comparable to a healthy lung. Asthma was modeled with IL-13 exposure, inducing goblet cell hyperplasia, hyper-secretion of cytokines G-CSF and GM-CSF, and decrease in cilia movement. Furthermore, a patient specific disease model was built with primary airway epithelial cells harvested from a patient with chronic obstructive pulmonary disease. This model mimicked pathogen infection with increased cytokine secretion and increased neutrophil recruitment under LPS or poly(I:C) stimulation. Therapeutic value was additionally demonstrated with testing of an experimental anti-inflammatory drug, 2-methoxy-*N*-(3-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide, which showed suppressed neutrophil recruitment and cytokine secretion, in agreement with prior finding from animal models. This model was recently expanded to study the risk factor of smoking in a healthy and a diseased state, an impressive demonstration that shows the potential of organ-on-a-chip systems to model the effect of environmental toxins on the human body.[89]

The use of PDMS in Emulate's system facilitates rapid prototyping. However, PDMS absorbs small hydrophobic molecules,[90] which could significantly affect the drug

concentrations in solution in light of micro-channels with high surface-to-volume ratio. This limits the use of PDMS-based devices in industry for drug development application. To overcome this, Dr. Ingber and colleagues developed a polyurethane based polymer that resists the absorption of small hydrophobic molecules while preserving the desirable qualities of PDMS, such as optical transparency, flexibility and castability.[91] Another potential alternative is styrene–ethylene–butylene–styrene (SEBS) copolymers, which have been shown to be easily castable, transparent, and elastic.[92–94] They are also biocompatible and most importantly do not absorb hydrophobic molecules. However, these alternative polymers are yet to be widely used in organ-on-a-chip devices in academia, but the use of SEBS to construct Emulate's devices has been shown in patent applications.[95]

The cell-to-liquid ratio is another important factor to address in the Emulate system. The volume of the circulating medium tends to far exceed the volume scale of the tissue. Therefore, the continuous dilution of secreted factors and metabolites has a significant impact on the cells, especially for devices with single-pass perfusion. The surface-to-volume ratio might be another consideration, especially when local paracrine and autocrine signaling is important. For instance, when modeling the micro-vasculature, even when microvascular endothelial cells are used, the size of the vascular chamber in these models would still far exceed the size of a capillary. Similar to Hespero, TissUse, and CN Bio, as Emulate attempts to connect the multiple organs together, the issue of cell-to-liquid ratio, scaling effects, and common medium will require further examination.

Another concern of using membrane based devices to model vascular and epithelial interfaces is that the thickness of the membrane (~10 μm) does not match the thickness of the basement membrane (300–400 nm) of blood vessels.[96] In fact, their thicknesses differ by 2 orders of magnitude. A method of overcoming this physical limitation is to mold hydrogels with micro-channels that can be coated with endothelial cells.[97] Since hydrogels are permeable and allow cell migration, pericytes embedded within hydrogel matrices could be recruited to the vessel interface and closely associate with the endothelial cells. Between the pericytes and endothelial cells a basement membrane is naturally deposited. While there is no shortage of hydrogel molding methods,[98] tissue vascularization[32,99] is a major limitation in producing thick tissue in tissue engineering. Microfluidic hydrogels may offer promising solutions to overcome this challenge. 3D bioprinting has made tremendous progress in this area by recreating the complex vascular and epithelial networks in 3D tissues with the sacrificial molding method being an especially popular choice.[100–104] Although not yet commercialized, in a number of demonstrations, we are beginning to see a convergence of 3D bioprinting with organ-on-a-chip,[103,105–107] leading to greater control and automation over tissue structure formation and analysis in organ-on-a-chip devices.

Building tissue models for drug discovery, however, does not require a large tissue. Primary interests reside in the vascular or epithelial interface generated within a hydrogel. Therefore, instead of a complex network, a single tubular structure might sufficiently simulate the mass transport dynamics of the vascular interface. With the backing from investors that included the Bill and Melinda Gates Foundation, Nortis (Seattle, WA) developed vessel-on-a-chip[108] and kidney-on-a-chip[109] products for drug testing. The commercialized platform utilizes a microfiber to mold a single straight channel (diameter ~100 μm) within a collagen I matrix. This molded channel is then coated with collagen IV to facilitate the attachment of primary human proximal tubular epithelial cells[109,110] to yield an engineered human kidney proximal tubule analog. When cultured on the platform, cells were seen to polarize according to basolateral expression of Na^+/K^+ ATPase. Perfusion was shown to improve cilia formation. The engineered tubules also demonstrated reclamation of glutathione, glucose reabsorption, responsiveness to pH change, and bioactivation of vitamin D. In a comparison between conventional Transwell® and the 3-D perfusion systems, both demonstrated the passage of an organic solute, para-aminohippurate, across the barrier. Uniquely, the 3-D perfusion system demonstrated a drop in permeability in the presence of a competitive inhibitor, probenecid, implying that key transporters, such as OAT1/3 and MRP2/4, were actively expressed.

In the Nortis system co-culture of pericytes and endothelial cells was also demonstrated.[108] Robust endothelial sprouting and pericyte interaction has been shown. In other hydrogel-based systems the interaction between pericytes and endothelial cells has also been demonstrated extensively, suggesting the potential to assemble a bi-layer vascular interface in a similar approach.[97,111,112] In the initial prototyping stage, Nortis' devices were made from PDMS. However, their device could be productized with tissue culture plastics. These promising results motivate further development of these chip based devices focused on increasing the throughput, minimizing the number of manipulations needed to perfuse a single device and eliminating inter- and intra-device variability that comes from varying perfusion rates and imperfections in hydrogels.

To improve the throughput of hydrogel-based devices, Dr. Paul Vulto and Dr. Jos Joore developed phaseguide-assisted patterning of a hydrogel within microfluidic channels to create hydrogel–liquid interfaces. Dr. Paul Vulto developed this technique initially for the purpose of RNA extraction,[113] but the same technique can be used to build a stratified 3-D cell culture system with alternating hydrogel and liquid perfusion lanes for modeling vascular and epithelial interfaces. Pivoting this technology to the emerging market of organ-on-a-chip, together Dr. Paul Vulto and Dr. Jos Joore founded Mimetas. They productized this technology in the format of a multi-well plate, named OrganoPlate™, where a hydrogel solution can be injected into a channel with micropipettes and patterned according to the phaseguide configuration. The liquid lanes are perfused by gravity driven flow on a rocker. However, some endothelial cells can be exquisitely sensitive to

reverse flow, hence there is still a need to develop one directional flow in a simple setup.

On Mimetas' plate cells can be embedded within the hydrogel, resembling the parenchymal space[114–116] or coated on the hydrogel surface to mimic the vascular or epithelial interface.[117] The system is versatile and allows the study of cell migration, biochemical gradient, 3-D co-culture, perfusion, barrier function, *etc.* Mimetas' OrganoPlate™ in a 384-well plate format is completely PDMS-free and is likely the most compact and high-throughput organ-on-a-chip system on the market. Organ-on-a-chip systems, due to their complexity, rarely reach a footprint of more than 100 devices on a single platform. In conjunction with standard high-throughput imaging techniques for multi-well plates, such systems could be adopted for early stage drug screening in addition to late stage drug target validation. However, the main advantage of organ-on-a-chip platforms is that they can provide high-content information on a variety of parameters, rather than high-throughput information on a single parameter.

Culturing of neurons,[116] hepatocytes,[114,115] endothelial cells, kidney proximal tubular cells,[117] cancer cells, *etc.* has been accomplished in the Mimetas system. The barrier function of endothelial cells and kidney proximal tubular cells was maintained for multiple days. The width of each channel compartment was kept narrow (around 0.2 mm) to allow sufficient molecular exchange between the vascular barrier and the parenchymal space.[115] Depending on organ type and location, higher vascular density might be necessary. For instance, in the myocardium each cardiomyocyte is in physical contact with a microcapillary.[118] Pericytes and endothelial cells were also co-cultured in the system to form a bi-layer vessel with endothelial cells covering the inner luminal surface. However, the stability of the vessel barrier depends on the stability of the hydrogel. The presence of large populations of parenchymal and stromal cells could accelerate hydrogel remodeling, alter the hydrogel/liquid interface and introduce variation in the system. With this in mind, it remains to be seen if this system could be further improved to model solid organs, such as the heart muscle, which has a physiological cell density as high as 10^8 cells per cm in the parenchymal space.

In an effort to simulate the physiological architecture of a microvascular network, SynVivo developed micro-chips embedded with micro-networks duplicated from images of actual tissue microvasculature. Founded by Dr. Kapil Pant and Dr. B. Prabhakar Pandian, SynVivo initially utilized the physiological microvasculature design to examine the spatial variation in the interaction of drug carriers, immune cells, and nanoparticles with the endothelium (productized as SynRAM).[119–129] Later, the company expanded to incorporate parenchymal tissues around the microvasculature with a multi-compartment design connected through a narrow wall patterned with micro-channels used for general toxicity screening (productized as SynTox). In this configuration, the interaction of tumor cells and astrocytes with the endothelium can be used to model the blood–tumor barrier (SynTumor)[130–132] and the blood–brain barrier (SynBBB) function,[133,134] respectively. The size of the micro-channels in the SynVivo devices is approximately 50–100 μm, which is above the average size of microvasculature and more closely resembles the post-capillary venules as is the case in many other attempts to vascularize tissues. However, with this microfabrication based approach the micro-architecture of the microvessels network can be robustly reproduced, maintained, and imaged[135] over long periods of time.

Taking advantage of the intrinsic ability of the endothelial cells to self-assemble into a microvascular network, 4Design Biosciences, founded by G. Wesley Hatfield, Christopher C. W. Hughes, Steven C. George, and Abraham P. Lee, is commercializing a vascularized micro-organ (VMO) platform chip that can perfuse a self-assembled microvascular bed. The device contains micro-chambers filled with endothelial cells embedded in hydrogel matrices and flanked by two perfusion channels.[136,137] The endothelial cells, which can self-assemble into a functional vasculature, will also connect with the perfusion channel and establish vascular perfusion. This approach creates micro-vasculature at a physiologically realistic dimension. Since the hydrogel matrix allows biological remodeling, the dynamic assembly, re-structuring, and deterioration of the vascular network can be tracked in response to biological and pharmaceutical inputs. Significant efforts were put in place to optimize the flow circuit resistance,[138] matrix composition,[139–141] and vascular anastomosis with the perfusion channels[142] to ensure robust operation of these devices. Furthermore, the device has now been productized in a multi-well plate format.[143] Although the ease of use in this format is a major advantage, the low-pressure gradient generated does result in a two to three times reduction in lower fluid shear stress in the microvascular network compared to physiological conditions. Additional parenchymal tissues, such as cancer cells, have also been incorporated along with the endothelial cells.[144,145] The freedom to allow vascular remodeling in the presence of the cancer cells makes this platform an ideal model to study the dynamic vascularized tumor environment. Located in Singapore, AIM Biotech is commercializing a similar micro-vasculature-on-a-chip platform,[146–151] based on the research of Dr. Roger Kamm. The multi-well plate based platform can be used to study vascular angiogenesis, migration, and cancer metastasis.[152] By applying an interstitial fluid pressure gradient across the vascular bed, the device was used to uncover a novel mechanism of cell migration in response to fluid stress and matrix adhesion.[153] More recently the platform has been expanded to model the blood–brain-barrier function.[154]

To this point, few of the commercialized organ-on-a-chip products have incorporated the smooth muscle cells around the vascular interface. Assembling this layered vascular structure to model vascular dilation and relaxation is technically challenging. Nevertheless, one of the first commercial organ-on-a-chip products, developed by Dr. Axel Guenther at the University of Toronto, is an artery-on-a-chip[155,156] device for

ex vivo culture of small diameter blood vessel explants. The technology was later licensed to Quorum Technology. Probing the structural and functional changes in small diameter arteries and veins *ex vivo* is important for the study of the pathogenic factors involved in cardiovascular diseases. Dr. Guenther's platform can load, culture and stimulate isolated small arteries[156] and veins for this function. Pneumatic suction channels within the device can hold the vessels in place and keep them open for perfusion. Drugs can be perfused through and around the vessel from both the luminal and the abluminal sides. Vessel dilation and constriction can be visualized and quantified on-chip against step-wise increase in drug dosage. Automated tissue staining is also possible on-chip. The devices were made of PDMS in the prototyping stage, but the device could be productized with industry plastic materials. This approach, however, is limited by the invasive surgical isolation of vessels, which minimize reproducibility and throughput. The system itself, however, has been integrated with an automated system to lessen user tasks. Moreover, there are currently no replacements for native small diameter vessels that can provide equally sufficient physiological function, specifically in the study of the endothelial barrier function coupled with physiological smooth muscle dilation and contraction.

The various dynamic tissue interfaces within our organs provide opportunities to develop complex organ-on-a-chip devices with sophisticated external control systems to precisely control fluid flow, mechanical movement, and molecular transport. Remarkable physiological hallmarks have been achieved in the emulation of these tissue interfaces as demonstrated in the Emulate systems. In academic research the excitement over organ-on-a-chip was significantly catalyzed by the introduction of the lung-on-a-chip device in 2010 (ref. 77) as seen by a remarkable rise in the number of scientific papers published in this area (Fig. 1). Following the membrane device paradigm, numerous groups have continuously adapted the technology to culture and study a broad range of cell types and biological structures, including endothelial cells,[157] placenta,[158] skin,[159] podocytes,[160] endometrium,[161] cancer cells,[162,163] eyes,[164] and the blood–brain-barrier.[165] Pairing this type of compartmentalized organ-on-a-chip device with analytical techniques, such as ELISA and mass spectrometry, is especially useful to assess the biological change of an individual cell type in a co-culture environment. However, the built-in complexity of these systems and the specialized hardware that is needed to run these devices in many cases make them inaccessible to non-experts.

Emulate is working toward productizing supporting instruments to minimize the technical complexity involved in chip operation as is Nortis. It remains to be seen what the cost of these automated integrated systems will be. We anticipate that the throughput of these systems will make them most suitable for later stage lead optimization and target validation phases of drug development, rather than a first screening tool for new chemical entities that requires a throughput on the order of 10 000 compounds per screen. On the other hand, companies like Mimetas, 4Design Biosciences, and AIM Biotech have focused on adapting their technology to a conventional multi-well plate format, which could be integrated into the existing infrastructure in pharmaceutical drug screening and biological analysis systems. Furthermore, the cell-to-liquid ratio is an important issue that has not received a lot of attention by companies that have focused primarily on single organ modeling, and dilution of secreted factors is an issue that needs to be addressed. For technologies that have adopted a smaller multi-well plate footprint that uses a small amount of medium (such as Mimetas, 4Design Biosciences, and AIM Biotech), the physiological cell-to-liquid ratio might be more easily achieved.

The tissue epithelial and vascular interface is an important component that makes up a functional tissue, but it could not represent the entire organ. As we move away from the various tissue interfaces and dive deeper into the parenchymal space, the physiological functions of many solid organs will increasingly depend on the structure and function of the parenchymal cells.

Parenchymal tissue-on-a-chip

The liver serves a number of vital physiological roles: carbohydrate metabolism, including the glucose regulatory mechanisms of glycogen storage and gluconeogenesis; lipid metabolism, including the synthesis of cholesterol and triglycerides; protein metabolism, including the synthesis of amino acids, albumin, insulin-like growth factor 1, angiotensinogen, the coagulation factors fibrinogen and prothrombin; and bile acid secretion. The liver is positioned as a front-line anatomical exposure to orally administered drugs and digested food (as well as any pathogens that penetrate through the mucosa) absorbed from the gastrointestinal tract *via* the portal vein. Hepatotoxicity is one of the first concerns in drug design as many enzymatic pathways of the liver, such as methylation, convert precursor drugs into metabolites that may be active, inactive or cytotoxic. One third of the drugs withdrawn from the market are attributed to liver toxicity. As hepatocytes can be conveniently expanded *in vitro*, primary human hepatocytes have been a popular cell source for applications in ADME/Tox. Unfortunately, primary hepatocytes lose their function rapidly in conventional culture systems after isolation from their native environment.[166] Therefore, stabilizing hepatocyte function *in vitro* has received significant attention.[44]

It was found that co-culture of hepatocytes and stromal cells appreciably improved the functionality and stability of hepatocytes *in vitro*. The benefit of this co-culture is realized with an optimal level of organization through micro-patterning, where hepatocytes were cultured in 500 μm islands with 1200 μm spacing surrounded by the stromal cells.[167] The balance of homotypic and heterotypic interactions plays a key role here, as the optimal ratio changes between cells of different species. These differences could be modeled accordingly by varying micro-pattern sizes. This form of micropatterned

co-culture system was pioneered as a stable monolayer culture in a multi-well plate format by Dr. Sangeeta N Bhatia and later commercialized as HepatoPac by Hepregen Inc. Hepregen optimized HepatoPac with cells of many species, thus providing the option of comparing their *in vitro* system directly against animal tests that have served as the benchmark for pre-clinical screening.

Since its founding in 2008, Hepregen has received funding from private investors and government grants, launching its first product (HepatoPac) in 2013. In a project carried out with Pfizer,[168] HepatoPac was used to test 35 known drugs that cause drug-induced liver injury and 10 control drugs that have no known liver toxicity.[169] Taking advantage of the long-term stability of the HepatoPac system, drugs were tested with repeat dosing (four doses over 14 days) rather than short-term exposure (one dose for 24 h) on a conventional collagen sandwich platform. The repeated exposure procedure was hypothesized to subject cells to multiple forms of injury to reveal overt cellular stress. The drugs with known liver toxicity were captured with 65% success, whereas 10% of control drugs gave a false positive. This result was an improvement compared to a 50–60% true positive rate and 0–5% false positive rate on the conventional platform,[169] demonstrating the advantage of repeat dosing.

Clinical hepatotoxicity can also be due to a combined usage of drugs such as didanosine and stavudine. In addition, the original system could not capture drugs that trigger allergic responses involving histamine and cytokine secretion by mast and basophil immune cells. To compensate for this, macrophage-like primary human Kupffer cells of the liver were added to the co-culture system to access proinflammatory cytokines.[170] The expanded intercellular cross-talk of this system permitted the detection of cytokine effects on metabolic enzymes and drug transporter genes of hepatocytes, as effected through non-hepatocyte cytokine-targeted receptors (*e.g.* IL-2 and IL-23). With a multi-well plate format and highly reproducible manufacturing process, HepatoPac exemplifies a product which works well in the hands of non-experts; Hepregen offers plates already seeded with cells. In the hands of a user, only standard tissue culture and analysis techniques are required. The democratization of this platform to generate user specific data is apparent from the number of publications on drug metabolism and depositions from various groups using the HepatoPac.[171–179]

Separate from the company, Dr. Bhatia continues to explore the dynamic regulation of cell–cell contact in a micromechanical reconfigurable culture system, where cells are cultured on two micromachined silicone substrates with comb finger-like structures. These can be locked into each other to bring the two cell types into contact on demand.[180] This work revealed that hepatocyte stability can be maintained with initial direct contact for hours followed by sustained soluble signaling. Extending the concept of the dynamic co-culture to a 3D environment, our lab also demonstrated that scaffolds with a hook-and-loop design allow 3-D assembly and disassembly of functional cardiomyocytes and cardiac fibroblasts on demand to build 3D functional tissues with spatially controlled heterotypic interactions.[181] For hepatocytes, the benefit of homotypic and heterotypic interactions is also translatable to 3-D cell culture. Dr. Bhatia and colleagues developed the "Intaglio-Void/Embed-Relief Topographic (InVERT) molding" method,[42] that enables placement of stromal cells around hepatocyte aggregates within a hydrogel system. This 3-D system, rooted in the importance of heterotypic interactions, was implanted subcutaneously in rats and demonstrated improved hepatocyte survival and function up to four weeks.

The ability of patterning cells in 3-D with a production process that is scalable is important in drug screening since this 3-D environment is more organ-like and will remove cells from contact with hard cell culture plastics. Organovo (ONVO), based on Dr. Gabor Forgacs' work at the University of Missouri, is the first 3D bioprinting company and one of the few publicly traded bioprinting companies.[182] Printing tissues or organs for surgical therapy and transplantation faces numerous scientific and clinical hurdles, but *in vitro* drug testing using 3D-printed tissues is considered a simpler technological space, for which the requirements on the generated tissues are less stringent. Organovo, which began as a bioprinting company, is now strategically aligning their products toward *in vitro* models for drug discovery. Leveraging their printing techniques, they developed ExVive3D™ Liver by directly depositing and patterning hepatocytes and nonparenchymal cells (hepatic stellate cells and endothelial cells) in 3D to form a liver module. This module was constructed with nonparenchymal cells surrounding hepatocytes with a final tissue thickness of 500 μm.[183]

The importance of balancing homotypic and heterotypic interactions justifies the printing and patterning of the parenchymal and nonparenchymal cell types in separated aggregates, instead of random mixing. Organovo's printer dispenses concentrated cell aggregates with diameters of 300–500 μm.[184] Achieving a high print resolution at the cellular level is not a major focus here. Instead, this approach focuses on maximizing cell viability and establishing high cell concentration, which is critical to the subsequent physiological remodeling. The tissue construct can be histologically sectioned for immunostaining and *in situ* hybridization, a standard clinical pathology lab procedure followed in the processing of patient biopsies. This thick 3D environment also successfully models biochemically induced liver fibrosis[185] which requires significant tissue mass to develop.

The liver modules generated here maintain ATP, albumin, and cytochrome P450 expression for at least four weeks in culture. As shown in one study[183] Organovo's liver model detected the toxicity of trovafloxacin, a drug withdrawn from the market due to hepatotoxicity not discovered in the preclinical stage. Building on ExVive3D™ Liver, Organovo is also expanding its portfolio to include a kidney proximal tubule model. This is driven by a perceived strong market need for renal toxicity testing and biomarker development. Early adopters, such as Ardea Biosciences, a subsidiary of

AstraZeneca, and La Jolla Pharmaceutical, have provided positive feedback and are integrating this product into their drug programs.[186] Working with L'Oreal, Organovo is also developing 3D-printed human skin for cosmetic screening and reconstructive skin grafts.

3D bioprinting companies in the organ-on-a-chip commercial space are not limited to Organovo. A Canadian based company, Aspect Biosystems, developed a Lab-on-a-Printer™ bioprinting platform that can dispense cells in an alginate based hydrogel carrier. From this 3-D printing technology, the company developed the 3DBioRing™ Airway tissue by printing airway smooth muscle cells into a ring-like structure in a dish to model respiratory functions and diseases such as asthma that are characterized by airflow obstruction.[187,188] The airway tissue model demonstrated contraction and relaxation and response to external physiological stimuli such as histamine, B2-agonist, *etc.* Interestingly, the company also claims the ability to freeze the 3DBioRing™ tissues for storage with maintenance of contractile and relaxation functions upon thawing. This is an important step towards the development of off-the-shelf organ-on-a-chip products. Other 3D bioprinting companies, such as Advanced Solution Life Sciences, BioBots, *etc.*, provide only 3D bioprinters, not any tissue models. Government agencies are also actively engaged in developing 3-D bioprinting. For instance, Brad Ringeisen of the Naval Research Laboratory (NRL) has developed the biological laser printing (BioLP) method that can dispense cells in high resolution without the use of any orifices.[189,190] Andre A. Adams from NRL developed extruded microfibers with coaxial flow in microfluidic devices.[191] Other review papers can be found to provide a more extensive overview of 3-D bioprinting.[41,192–194]

In most organs, cells are never in contact with any hard plastics. But *in vitro*, this is difficult to avoid since tissue culture materials are almost universally molded from thermoplastics like polystyrene, PET, PMMA, and COC. InSphero reinvented the conventional multi-well plate by removing the plate base so that culture media can form a hanging drop at the plate bottom, supported by surface tension. Cells distributed in culture media readily settle towards the hanging drop and form a spheroid inside the drop. This design facilitates 3D culture by allowing single cells to spontaneously grow into 3D aggregates. Tissue spheroids present an ideal geometrical format for culturing solid organs, such as the liver and pancreas, and tumors. The cell aggregates can avoid contacts with any hard plastics and form a more physiological environment. However, cell patterning within the spheroids is limited to random mixing. Nevertheless, co-culture of lung cancer cell line A549 with fibroblasts in spheroids showed self-directed spatial organization with fibroblasts located predominantly in the spheroid core.[195] Such interaction between the tumor and the stromal cells appears to be highly cell-line dependent. Tumor spheroids from bone cancer cell-lines SaOS-2 and HOS showed a general drug resistance with increased IC_{50} compared to 2-D culture, suggesting an additional factor in drug penetration to be considered in 3-D tissue spheroids. In another case, tissue spheroids from breast cancer cell-lines showed enhanced growth inhibition when treated with a combination of radiation and drug (trastuzumab) therapy when compared to 2-D culture.[196] The hanging drop assembly method was sufficiently versatile to generate hepatic,[197,198] pancreatic[199] and cardiac spheroids.[200,201]

Furthermore, it is important to note that the hanging drop technology, initially introduced to assemble tissues in multi-well format, has since been upgraded with microfluidic circuits through the academic effort of Dr. Oliver Frey at Eidgenössische Technische Hochschule Zürich. Endowed with flow circuits, this technology was transformed into a dynamic culture system, allowing for a dynamic nutrient supply, drug dosage and metabolic communication between multiple tissue spheroids.[65] For instance, a microfluidic gradient generator was devised to quickly generate a series of drug dosages applied to an array of tissues, and a dose response curve was derived from a single chip. Microfluidic pneumatic valves were also incorporated to generate closed-loop pulsatile media perfusion. In an elegant demonstration[201] the contraction of a cardiac spheroid cultured in the system was imaged and recorded as the beating profile was fed back to an external pneumatic controller to generate a synchronous pulsatile flow pattern that matched the beating of the cardiac spheroid. Thus the feedback control system allows the physiological effect from the beating heart to indirectly drive the flow circulation of the entire culture system. However, it does complicate the setup and reduce throughput, offsetting the advantage of the hanging-drop technology in its original multi-well format. The missing capability to apply forces, electrically stimulate to produce synchronous contractions and measure forces is also significant in the cardiac field.

To harness the benefit of perfusion while maintaining a simplified setup, the hanging-drop technology was reconfigured with gravity driven flow.[202] The perfusion capability allowed for co-culture of liver and colorectal tumor spheroids. Up to 48 devices were cultured in parallel, demonstrating the robustness and scalability of the setup. The prodrug cyclophosphamide, which inhibits tumor growth but only with bioactivation by the liver, showed efficacy in this perfusion based duo-organ culture system. In static culture, this is not reproduced; the transfer of conditioned media from cyclophosphamide-treated liver spheroids to tumor spheroids resulted in no growth inhibition. This result substantiates the importance of dynamic perfusion in maintaining a more realistic liquid-to-cell ratio and accelerating biomolecular exchange for multi-organ co-culture. Tissue spheroids are a popular tissue culture format[203] which can also be generated with a variety of other methods, such as the use of a non-adherent Petri dish,[204] AggreWell™,[205] and droplet microfluidic devices.[206] 3D Biomatrix also provides similar hanging-drop plates. Several other publications also describe tissue spheroid culture in microfluidic devices in more detail.[207–209]

One important benefit that recirculating perfusion brings in liver tissue culture is the ability to more accurately model drug clearance and to approach physiological cell-to-liquid ratio. Hurel Corporation, founded by Dr. Greg Baxter and Dr. Robert Freedman, is commercializing a microfluidic biochip that allows for the continuous recirculation of culture media over a hepatocyte monolayer to predict hepatic clearance.[210] The device has demonstrated more predictive clearance values and greater metabolite generation than static culture.[211] Similar to Hepregen, the company offers consumers hepatic co-culture models of human, dog and rat species in standard multi-well plate format.[212] Working with multiple pharma companies, the co-culture platform has been characterized in depth and allows robust repeat drug dosing. Leveraging expertise in microfluidic cell culture technology, Hurel is also working with L'Oreal to develop an Allergy Test-on-a-Chip™ by linking an artificial lymph node construct with a skin construct.[213] The duo-organ system, separated by microfluidic channels, is designed to maintain chemical gradients across the two organs and can drive the migration of immune cells such as the dendritic cells or T cells for the study of allergic reactions.

Furthermore, through the combination of perfusion and co-culture, KIYATEC developed the 3DKUBETM™ system that provides an *in vivo* like tumor environment to derive drug response profiling (DRP) and predict *in vivo* response. Notably, KIYATEC uses patient-derived xenograft (PDX) tissues or clinically sourced primary cancer tissues, yielding more clinically relevant results. This heterotypic microtumor contains adipocytes, fibroblasts/epithelial cells, and cancer cells in Matrigel™ and Collagen I. KIYATEC has also placed a significant effort in creating patient-matched immuno-oncology models by incorporating tumor-associated macrophages (TAMs), T-cells, and cancer-associated fibroblasts (CAFs). For instance, the company demonstrated that M2 macrophages support tumor metabolism and present an immunoprotective effect *in vitro*, and contrasted these results with the M1 phenotype.[214] Furthermore, they demonstrated the co-culture effect on tumor cell viability; $CD3^+$ immune cells had a negative impact, whereas $CD14^+$ cells had no impact.[215] The assessment of noninvasive optical assessment of redox ratio from perfusate, a measurement of cell metastasis potential, revealed physiological drug effects and the significance of environmental factors, such as perfusion and 3D co-culture, in maintaining tumor functionality.

Co-culture of multiple cell types at the right ratio and at the right time is at the core of simulating the complexity of the human immune response. With this strategy, The Modular Immune *In vitro* Construct (MIMIC) Technology from VaxDesign of Sanofi Pasteur models the innate immune response of peripheral tissue and the lymphoid tissue.[216–218] The platform, initially developed with DARPA funding from 2004 to 2009, is very simple and is compatible with high-throughput screening. It contains a monolayer of endothelial cells (HUVEC) seeded on a collagen matrix. But when human immune cells from donor blood are placed on the monolayer, cells like monocytes will transmigrate across the endothelium and autonomously convert into antigen-presenting cells in the collagen matrix; some of which will migrate back to the endothelium. The antigen-presenting cells (dendritic cells) can then be stimulated with antigen, vaccine, and immunogen in the culture media to generate an immune response. The dendritic cells can be further co-cultured with T cells and B cells at the right ratio and right time to simulate the sequential activation of T cells and B cells, hence reproducing the environment of the lymph node. This technology coupled with a blood bank of a large diverse donor population can successfully predict the effectiveness of a vaccine over a large population, essentially a "Clinical Trial in a Test Tube™".

Moving from a planar interface of endothelial–epithelial cells, the Emulate technology can also be used to build increasingly complex biological microenvironments such as bone marrow-on-a-chip.[219] The complexity of the hematopoietic niche in bone marrow has never been recapitulated *in vitro*. To accomplish this, a PDMS device containing a cylindrical cavity filled with Type I collagen, demineralized bone powder (DBP) and bone morphogenetic proteins (BMP2 and BMP4) was implanted subcutaneously in rats to recruit hematopoietic cells for eight weeks. The populated bone marrow disk was then explanted and placed in the membrane-based device under perfusion culture for at least one week in the presence of hematopoietic cells and progenitor cells in physiological proportion. The engineered bone marrow was challenged with γ-radiation and demonstrated the protective effects of radiation countermeasure drugs and granulocyte colony stimulating factor (G-CSF). The use of this type of implanted bioreactor is not new,[220,221] but the transfer of bone marrow graft from an *in vivo* bioreactor back to an *in vitro* one is novel. This study indicates the potential uses of organ-on-a-chip *in vitro* systems for the *ex vivo* culture of clinical biopsies and explants in addition to modeling complex biological environments or structures that would otherwise be difficult to recapitulate.

Culturing liver tissues and cancer cells in spheroid format is a popular approach as these tissues assume spherical morphology *in vivo*. However, for cardiac or skeletal muscles whose functional morphology is elongated, a tissue fiber format is a more appropriate choice.[32] Generating tension within an assembled tissue is important to forge properly elongated cardiac or skeletal muscles *in vitro*. To provide this tension, one may embed anchored physical structures in various configurations, such as a flexible sheet,[222] parallel posts,[223,224] parallel rods,[225,226] post array,[227–230] a single wire,[231,232] parallel wires[233,234] or a mesh structure.[181,235–237] Each of these structures constrains and guides the stretched tissue morphology. Cardiac tissues uniquely respond to both mechanical and electrical stimulation and such external cues play a significant role in cardiac tissue maturation. We demonstrated previously the use of electrical stimulation in maturing engineered tissues from primary rat cardiomyocytes[238] in 2004. The conundrum faced in the engineering of cardiac

tissue models is that primary human cardiomyocytes cannot be easily obtained in large quantity from human donors, and at the same time terminally differentiated cardiomyocytes are non-proliferative, and thus cannot be expanded.[239] Therefore, a reliable source of cardiomyocytes was urgently needed. Although pluripotent stem cells such as embryonic stem cells can give rise to cardiomyocytes through spontaneous differentiation, the yield (~2%) was too small for practical use.

In 2008, Dr. Gordon Keller (McEwen Centre for Regenerative Medicine, Toronto) developed a directed differentiation protocol achieving a high yield of cardiomyocytes from differentiating embryonic stem cells.[240] Newer protocols followed from the Palecek[241] and Burridge and Wu laboratories,[242,243] ultimately enabling a reproducible differentiation with a yield of cardiomyocytes of over 90%. In an effort to demonstrate the robustness of the differentiation protocol, differentiated cardiomyocytes from multiple universities were compared and were shown to display similar calcium handling properties.[244,245] These breakthroughs allowed researchers to obtain cardiomyocytes from both embryonic and induced pluripotent stem cells more efficiently and broadened their practical use in research. Furthermore, more specific cardiac subpopulations were derived from iPSCs, such as epicardial cells,[246] atrial-like cardiomyocytes,[247] and sinoatrial node cardiomyocyte cells,[248] opening the possibility of modeling specific aspects of cardio-physiology. However, the cardiac cells resulting from these differentiation protocols are generally considered immature. For instance, the cardiomyocytes display immature sarcomere structures (absence of H zones, I bands and M lines), immature action potentials, fetal-like calcium handling and gene expression.

During development, the fetal heart beat increases gradually to 3 Hz over the 2nd–3rd month and subsequently reduces in the middle of pregnancy.[249,250] Cardiomyocyte beating rate is closely connected to the expression of contractile proteins; hence the change in heart rate during development could be a fundamental mechanism of heart maturation. Our group demonstrated that differentiated cardiomyocytes could be matured by assembling them into an elongated tissue fiber (referred to as Biowire™) and cultured under electrical stimulation with increasing stimulation frequency from 1 Hz to 3 Hz or 6 Hz over one week.[231,251] This maturation process significantly improved the tissue myofibril organization, conduction velocity, electrical physiology, and calcium handling properties. Although the maturation state of these tissues is not yet adult-like (*e.g.* low membrane conductance), both structural and functional properties of the cardiomyocytes exhibited clear signs of maturation.

Based on the Biowire™ technology and the use of electrical stimulation for cell maturation, Dr. Milica Radisic and Dr. Gordana Vunjak-Novakovic together founded the company TARA Biosystems in 2014 to commercialize the platform for cardiovascular drug testing. The Biowire setup and the electrical stimulation apparatus was relatively simple to scale to a standard 96-well plate format that is also PDMS-free. Commercializing this technology in the conventional multi-well plate format will reduce translational costs and improve its adoption. TARA Biosystems is working closely with a number of pharmaceutical companies to validate its technology with arrays of clinically relevant drugs. With the backing of Harris and Harris, Alexandria, and the New York Partnership fund, the company established commercial operation in New York. The goal of the company is to produce Biowires economically on a larger scale and to provide service to drug companies to improve the reliability of their pre-clinical testing.

On a different front, the Radisic lab was also working to tackle the challenge of tissue vascularization for solid organs grown *in vitro*, as building a vascular network within a dense functional heart muscle is not an easy task. Heart tissue contracts continuously and distorts the tissue with each beat, so a built-in vessel network must possess stability to support repetitious dynamic strain with permeability for delivery of drugs and nutrients. Building on the advance in microfabrication and biomaterials, we developed AngioChip,[252] a synthetic polymer scaffold with a built-in micro-channel network rendered permeable with the micropatterning of microholes and nano-porosity on the channel walls. The stable scaffold provides control over the spatial arrangement of the parenchymal tissues and the internal vessel network. It also ensures that any tissue remodeling and dynamic movement happening within the parenchymal space does not affect the internal vessel network perfusion. Liver and cardiac tissues engineered in the AngioChip platform were able to process clinically relevant drugs. The engineered vascularized cardiac tissue contracted macroscopically and was implanted with direct surgical vascular anastomosis. For the purpose of *in vitro* drug testing, the AngioChip technology can be further miniaturized to fit into a standard multi-well plate. In this format, the AngioChip technology has been licensed to TARA Biosystems and could become the second-generation product after Biowire commercialization.

With a similar approach that takes advantage of the stability of synthetic polymers to build a vascular interface analog adjacent to an engineered cardiac tissue,[253] Dr. Kevin Healy developed a perfusable cardiac tissue chip. The chip has perfusion channels and a tissue chamber separated by a channel wall patterned with micro-channels (2 μm tall) to facilitate molecular transport across the wall. Its channel walls provide a stable interface to facilitate the culture of cardiomyocytes at high cell density within the microfluidic channels while allowing perfusion delivery of media or drugs from the perfusion channels. The width of the tissue chamber was around 200 μm, recapitulating the spacing of micro-vessels within the native myocardium. Modularizing this design, multiple devices (referred to as μOrgano) can be connected and disconnected on demand to simulate multi-organ interaction.[254] Since the system does not include a bubble trap, special care is needed to ensure bubble-free connection. In the prototyping stage, the devices were made from PDMS, which could be replaced with tissue culture plastic in future production. Advanced micro-structures and micro-electrodes have also

been incorporated to directly probe the electrophysiological responses of the tissues. This platform is being commercialized through Organos, Inc., founded by Dr. Healy.

The ability to measure the contraction force from the engineered cardiac tissues is one important physiological readout. Multiple approaches have been introduced to accomplish this. Cardiomyocyte cell sheets cultured on flexible cantilever-like structures allow the force of cell contraction to be measured from the bending of the cantilever.[105,255] The degree of bending can be tracked through either optical[255] or electrical[105] setups. External force probes can also be used to directly engage the tissue to detect functional changes, as demonstrated with the I-wire heart-on-a-chip platform developed by Dr. John Wikswo and colleagues.[233,234] An important benefit of using an external force probe is the ability to mechanically stretch the tissue while also tracking changes in active forces. This is particularly relevant to understanding the contraction force of the heart muscle in response to the loading of the heart chamber and recapitulating the Frank–Starling force–tension relationship.

Devices based on vertical posts have also been used extensively to measure tissue contraction forces from the bending of the posts based on beam bending equations. Wolfram Zimmermann and Thomas Eschenhagen pioneered this approach to induce cardiac tissue alignment by guiding tissue alignment through compaction of a collagen matrix between two posts.[256–258] The resulting matrix tension contributes critically to the making of high density aligned cardiac tissue bundles. The posts can also be mechanically stretched[35,226,258,259] or fitted with metal rods that increase the mechanical strength to simulate increased afterloads in the heart.[34] Eschenhagen and colleagues demonstrated that both cases can induce cardiac hypertrophy, which could be used as disease models. Microvasculature could also be incorporated into the heart tissue by spontaneous assembly of primary endothelial cells,[260] although it could not be perfused. However by micro-molding with an alginate fiber that functions as a sacrificial template for making a hollow channel within a compacted cardiac tissue,[261] the team was able to demonstrate improved tissue function with perfusion. Zimmermann and Eschenhagen commercialized their technology through the start-ups, Myriamed and ETH-Technologies, respectively. Automatic software and image acquisition apparatus were developed to detect tissue contraction force, frequency, and rhythm.[262,263] The elastic nature of the posts necessitates PDMS, which has the tendency to absorb small hydrophobic drugs, but could be replaced with other elastic materials in the future.

Cell alignment and guidance are also crucial for neuronal culture. AxoSim is commercializing their Nerve-on-a-Chip™ technology, based on the work of Michael Moore at Tulane University.[264–266] Their dual hydrogel systems, situated in a standard Transwell™ plate format, guide the neurite growth of a dorsal root ganglion tissue explant. The resulting neurons show distinct dendrite and neurite regions, which is amenable to clinical nerve compound action potential (CAP) and nerve fiber density (NFD) tests *in vitro*.[267–269] These clinical tests, performed for the first time on a purely cellular *in vitro* model, are of great value for the study of acute and chronic exposure to drugs of interest. AxoSim initially utilized rat tissue explants, but it is not clear if this has been expanded to human tissues. Xona Microfluidics, built on the work of Dr. Noo Li Jeon, developed a PDMS device with two parallel perfusion channels connected with narrow microgrooves.[270] When cultured with neurons, this device can compartmentalize the cell body and axon using the narrow grooves. The individually addressable compartments even allow sub-cellular axotomy (selective removal of axons). Expanding on this technology, MicroBrain BT, founded by Dr. Bernadette Bung, modified the design of the microgrooves with asymmetric width to achieve directional axon growth.[271,272] This important design modification allows unidirectional axon connection of two neurons in the separated perfusion chambers, hence establishing a complete neuron network. Ananda Devices, based on the work of Dr. Margaret Magdesian, is commercializing a similar silicon insert that can be used to pattern neurons and direct axonal extension.[273] However, the inserts will be removed at a later time point during culture, exposing the neurons. Coupling this patterning technique with atomic force microscopy further enables the user to control individual neurons and rewire the neuronal network manually.[274,275] In academia several sophisticated patterning techniques, such as the work of Molly Shoichet[276] and others,[277] also demonstrated guidance of neuronal growth in 3D *in vitro* culture. More extensive discussions can be found in other reviews.[278]

Current organ-on-a-chip models tend to examine the tissue interface and the parenchymal tissue separately. Companies that provide models of solid tissues (Organovo, Aspect Biosystems, Hepregen, and Insphero) permit sufficient tissue structural remodeling in their systems to build realistic functional tissue at high physiological cell density. This dynamic structural change during the remodeling step is critical to establish the proper intercellular junctions for the parenchymal cells. For companies whose products focus on modeling the vascular and epithelial interface (Emulate, Nortis, and Mimetas), any structural change and alteration at the tissue interface would be undesirable. In this aspect, our AngioChip technology is particularly useful in modeling both vascular interface and parenchymal tissue simultaneously, especially considering the opposing engineering criteria involved in establishing a functional parenchymal tissue and a stable tissue interface.

It is important to note that one of the advantages in organ-on-a-chip technology is its compatibility with existing imaging techniques. 3D tissues are inherently more difficult to image, especially if internal vascular interface must be visualized through a thick parenchymal tissue at physiologically relevant high cell density. As we build tissues that are increasingly realistic, there will be a greater need to develop built-in electronic sensors to probe tissue response in a non-invasive and high throughput fashion.[279–283] Recent advances

in microfabrication and 3-D printing have allowed for the direct incorporation of electronic sensors to track the electrophysiological and functional contraction of engineered cardiac tissues,[105,284,285] moving the field away from the low throughput image analysis methods.[105] To scale up and standardize chip production, lab-on-a-chip manufacturing companies, such as Micronit Microtechnologies, Microfluidic ChipShop, uFluidix, and many others, are partnering with organ-on-a-chip companies in the development of hardware and ramping up of production volume.

Patents

At the start-up stage, the competitiveness of many organ-on-a-chip companies relies heavily on the intellectual property (IP) licensed or owned by the company. One of the earliest organ-on-a-chip patents was filed by Cornell Research Foundation in 2002, which will be protected for 20 years till 2022, and it was licensed by Hurel Corporation. This US patent, based on the earlier work of Dr. Shuler, is very broad and essentially covers any perfusion based device that contains interconnected cell-culture chambers simulating multi-organ interactions.[286] A similar European patent from the same group has an even broader claim set that covers perfusion-based single micro-chamber devices for cell culture under conditions of *in vivo*-like pharmacokinetics.[287] Another important patent, filed by the Children's Medical Center Corporation in 2009 and based on the work of Dr. Ingber, is an organomimetic device with cells cultured on a stretchable membrane.[288] Prior to this, in 2003 Vanderbilt University filed a patent on perfusion bioreactors with a porous membrane for cell-culture, based on the work of John Wikswo.[289]

MIT also holds a patent filed in 1999, based on the work of Dr. Griffith, that broadly claims a system with a perfusable matrix containing one or more channels seeded with parenchymal cells and endothelial cells, where the endothelial cells assemble into a perfusable microvasculature. The use of a 3D printer to construct a biological model is also becoming increasingly important in the field of organ-on-a-chip engineering. Organovo licensed one of the broadest patents in 3-D bioprinting, which was filed by University of Missouri based on the work of Dr. Gabor Forgacs. The patent essentially claims a method of producing 3D tissue by arranging cell aggregates in specific patterns and then allowing them to fuse overtime.[290] Later this method was expanded with another patent that claims a method of using filler bodies, such as sacrificial materials, to assemble void spaces in tissue, applicable for tissue vascularization.[291] We understand that the patent field on organ-on-a-chip is enormous; hence these are some examples of the earlier patents and the overview is not meant to be comprehensive.

Since organ-on-a-chip devices can be easily reverse engineered, the trade secret is usually not a viable option for protection. However, many specific tissue compositions, culture media compositions, hardware, software, and technical tricks can be protected as trade secrets if the technology is not published academically. Furthermore, patent applications are also not disclosed initially for a long period. Hence it is not always easy to acquire updates on the current development of a private company.

Towards person-on-a-chip

Tailoring organ-on-a-chip devices to specific patient is a powerful aspect of this technology and aligns well with the current paradigm shift towards personalized medicine in healthcare. iPSCs[292,293] from patients harboring genetic cardiac mutations have been generated and differentiated into cardiomyocytes. These include cells from Timothy,[294] long QT,[295] LEOPARD,[296] and Barth[297] syndrome patients as well as dilated cardiomyopathy patients.[298] The use of cells with a genetic mutation in organ-on-a-chip devices could model genetic diseases with altered tissue level functions. These types of models will help accelerate the discovery of therapeutic treatment to these diseases. To facilitate this effort, large libraries of cells from patients with genetic diseases or healthy individuals have been generated in Europe (*e.g.* the Wellcome Trust, UK) and in the US (National Institutes of Health and California Institute for Regenerative Medicine).[299] Recent breakthroughs in gene editing[300,301] could further facilitate this effort by allowing multiple genetic diseases to be derived from a single healthy stem cell line.

Future perspective of organ-on-a-chip devices

The journey of a drug through the human body will involve all three aspects of human physiology: processing through multi-organ metabolism, crossing through tissue interface barriers, and stimulating parenchymal tissue response. Most organ-on-a-chip systems model only one or two aspects at a time, and it remains to be seen if one platform will emerge as a model of all three aspects of human physiology. Since each company presently holds only a piece of the entire puzzle, achieving this goal might motivate multiple companies to join forces to combine their techniques and patents, thereby fostering the continued evolution of more advanced products.

In academia, we are already seeing ambitious efforts to link technologies from multiple different labs (*e.g.* Gordana Vunjak-Novakovic, Sangeeta Bhatia, Christopher Chen and Karen Hirschi). With the support of a collaborative grant, their aim is to build integrated heart–liver–vascular systems derived from a single line of human pluripotent stem cells[302] with a focus on tissue maturation and incorporation of vasculature to achieve multi-tissue integration. Merging of various technologies also does not mean each technology must be physically linked. In a recent effort, a multi-organ model was established by transferring drug metabolites in media effluent of different organ models located in six different universities.[303] The study successfully demonstrated

drug absorption by the intestine, metabolism by the liver, secretion by kidney proximal tubules, transport across the blood–brain barrier, and toxicity on skeletal muscle myobundles *via* an *in vivo*-like sequential, inter-organ media transfer. This strategy allows the media flow rate in each organ to be adjusted individually per functionality. The effluent media composition can be further adjusted prior to entering the downstream organ module. This work revealed the potential of proper functional coupling to relax the need for strict tissue scaling in a physically integrated multi-organ system.

As the technology continues to advance, product offerings will likely overlap more significantly. Direct comparison between different technologies and against the current industry standard will become more imperative, which is currently limited by differing formats involved in each technology. To facilitate performance comparisons, there is an urgent need to define a series of physiological hallmarks for each organ and to establish a panel of clinically relevant model drugs against which to gauge each product. Regulatory agencies (*e.g.* Food and Drug Administration, FDA) have begun seeking improved testing methods[304] in collaboration with the pharmaceutical industry to develop industry standards.

One example of such initiatives is in the cardiac field, where a consortium is focused on developing the Comprehensive *in Vitro* Proarrythmia Assay (*i.e.* CIPA Initiative). The goal of CIPA is to enhance the accuracy of the cardiac proarrhythmic risk assessment for existing and new drugs. Similarly, in the liver field the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) representing the pharmaceutical and biotechnology industries has established the IQ Microphysiological Systems Working Group to develop standards for organotypic and microphysiological liver platforms.[305] Furthermore, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has initiated the Drug-Induced Liver Injury Network (DILIN) to establish a nationwide database to further delineate the association of severe liver injury with prescription drugs, over-the-counter drugs, and alternative medicines. Lastly, the American Institute for Medical and Biological Engineering (AIMBE) and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) at NIH have jointly organized annual workshops since 2012 to address the topic of Validation and Qualification of New *In Vitro* Tools and Models for the Pre-clinical Drug Discovery Process. The workshops aim to develop new guidelines on validation of new technologies for pre-clinical drug development.

There is still a long way to go for the organ-on-a-chip model to become fully integrated into the current drug development pipeline. However, the field has evolved well past the proof-of concept stage and is poised to prove its value through the demonstration of realistic physiological hallmarks and validation against standard drugs. The potential to model disease and predict human response to model drugs through this novel form of mechanistic human experimentation is enormous.

Conflict of interests

B. Z. and M. R. hold equity in TARA Biosystems Inc. This review describes the technologies behind the current start-up companies related to organ-on-a-chip that we are aware of. Since this is a dynamic area, new entities are emerging regularly and may have been missed in this review. Furthermore, the analysis of each technology and commercial entity could be non-uniform due to the availability of public disclosure.

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