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# Microengineered physiological biomimicry: Organs-on-Chips†

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Microscale engineering technologies provide unprecedented opportunities to create cell culture microenvironments that go beyond current three-dimensional *in vitro* models by recapitulating the critical tissue–tissue interfaces, spatiotemporal chemical gradients, and dynamic mechanical microenvironments of living organs. Here we review recent advances in this field made over the past two years that are focused on the development of ‘Organs-on-Chips’ in which living cells are cultured within microfluidic devices that have been microengineered to reconstitute tissue arrangements observed in living organs in order to study physiology in an organ-specific context and to develop specialized *in vitro* disease models. We discuss the potential of organs-on-chips as alternatives to conventional cell culture models and animal testing for pharmaceutical and toxicology applications. We also explore challenges that lie ahead if this field is to fulfil its promise to transform the future of drug development and chemical safety testing.

## Introduction

Understanding human pathophysiology requires investigation of how living cells and tissues function in the context of whole living organs. Organs are composed of different tissue types (*e.g.*, blood vessels, immune system, nerves, lymphatics) organized in distinct three-dimensional (3D) arrangements. Tissues, in turn, are composed of groups of cells linked together by extracellular matrix (ECM) scaffolds and cell–cell junctions. In our bodies, cells also experience organ-specific dynamic variations in spatiotemporal chemical gradients and mechanical forces (*e.g.*, cyclic strain, compression, fluid shear stresses) in their local tissue microenvironment that are crucial governors of their survival, growth, and function. Despite considerable technological advances, existing 2D and 3D culture models still fail to fully recapitulate these subtle organ-specific variations in the *in vivo* microenvironment. As a result, analysis of normal physiology and diseases processes commonly requires use of animal models, which are costly, slow, and questionable ethically; of even greater concern, is that they often fail to predict responses in humans.

The limitations of existing cell culture and animal studies have provided an impetus for the development of alternative cell-based *in vitro* models that better mimic the complex structures

and functions of living organs. Considerable advances have been made in this area as a result of the application of microsystems engineering for studies with cultured cells. Microfabrication techniques first developed to manufacture computer microchips have been adapted to enable precise control of cell shape, position, function, and tissue organization in highly structured 2D and 3D cell culture scaffolds.<sup>1</sup> Integration of microfabricated substrates with microfluidics technologies that enable precise control of dynamic fluid flows and pressures on the micrometer scale also has made it possible to create cell culture microenvironments that present cells (established human cell lines, primary cells or stem cells) with appropriate organ-relevant spatiotemporal chemical gradients and dynamical mechanical cues, which can induce cells to express a more differentiated, normal phenotype.<sup>2,3</sup> These new capabilities that emerged from the convergence of microengineering with microfluidics and cell biology have led to development of ‘Organs-on-Chips’ that reconstitute the structural tissue arrangements and functional complexity of living organs using cells cultured in microfluidic devices with relevant microarchitecture and microenvironmental signals. These microfluidic organs-on-chips permit study of diverse biological processes in ways that are not possible using conventional 2D or 3D cell culture systems, or even animal models.<sup>4–6</sup>

In this article, we provide an overview of progress made in this field over the past two years with a focus on the development and application of microengineered organomimetic microsystems for study of whole organ function and disease *in vitro*. We review engineering design principles and fabrication approaches that have enabled the creation of these biomimetic microsystems, which recreate minimal functional units of living organs that replicate their key structures and integrated functionalities. We also discuss the potential use of these ‘Organs-on-Chips’ for

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biomedical, pharmaceutical, and environmental safety testing applications, as well as challenges for the field that must be overcome to translate these technologies into useful products in the future.

## Reconstituting tissue–tissue interfaces

Early efforts to microengineer cell culture systems focused on microfabricating adhesive substrates that provide controlled microenvironments for control of cell shape, position, growth, and expression of differentiated tissue-specific functions.<sup>7,8</sup> As defined here, ‘differentiation’ refers to a process in which a cell or tissue undergoes structural and/or functional changes so that it exhibits specialized properties that more closely resemble the behaviors of similar cells within living tissues *in vivo*. Advances over the past decade in soft lithography-based microfabrication and microfluidics have made it possible to develop more sophisticated cell culture environments that recreate the complex 3D microarchitecture of living tissues and organs. For example, during the past two years, replica molding techniques – the transfer of topographical patterns from a microfabricated ‘master’ substrate into another deformable material to form an inverse mold – have been used to build a poly(dimethylsiloxane) (PDMS) microdevice containing microfabricated structures that mimics the structural complexity of the endothelial–epithelial interface that forms the liver sinusoid (Fig. 1).<sup>9</sup> The geometry of this engineered tissue interface was optimized to approximate the blood flow rate of the liver and to align rat hepatocytes (liver

epithelial cells) as they do along the endothelium-lined sinusoidal barrier *in vivo*. Interestingly, simply reconstituting the micro-architecture of this tissue–tissue interface was sufficient to induce cultured hepatocytes to self-organize into hepatic cord-like structures and form functional bile canaliculi *in vitro* even in the absence of living endothelium.

A simplified kidney model for analysis of kidney transport barrier functions also was created by stacking two microfabricated PDMS chambers, separating them using a thin porous membrane, and then culturing rat renal tubular epithelial cells on the upper surface of the membrane (Fig. 1).<sup>10</sup> Culture of kidney cells in the device under physiologically relevant levels of microfluidic flow greatly enhanced tissue polarization and induced formation of fully differentiated and polarized kidney epithelium. Laser ablation and hydrogel-based sacrificial replica molding techniques were used in another study to produce microscale 3D collagen scaffolds that replicate the geometry of human intestinal villi.<sup>11</sup> Culture of human Caco-2 intestinal epithelial cells on these scaffolds produced 3D epithelial structures with morphologies similar to those exhibited by villi of the human jejunum; however, the functional relevance of this response remains to be determined. A similar approach based on PDMS replica molding was used to create a microfabricated breast model.<sup>12</sup> This system consists of a network of branched microchannels lined with polarized human mammary epithelial cells that mimics the ductal system of the human mammary gland with the goal of developing a more physiological *in vitro* model for testing new breast cancer detection methods and

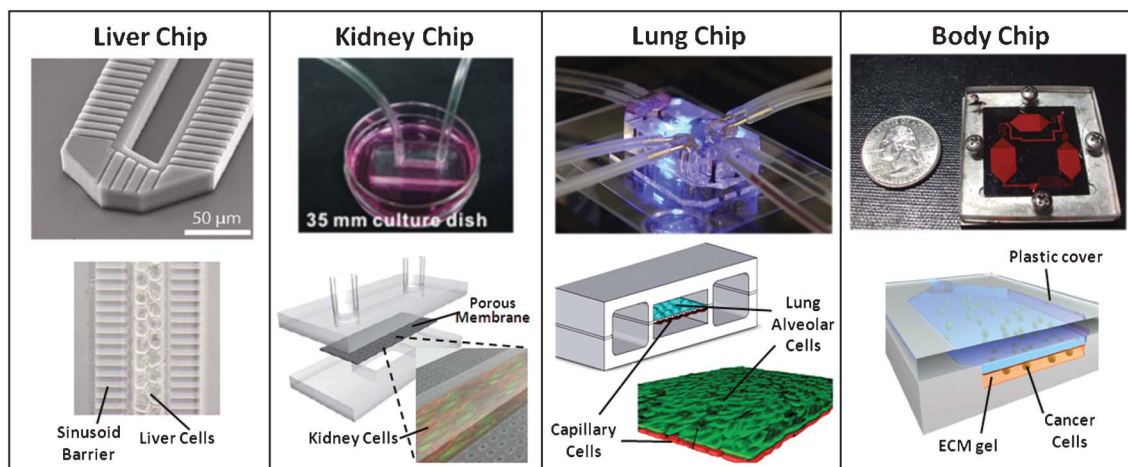


**Fig. 1** Mimicking tissue–tissue and organ–organ interfaces in organomimetic microdevices. **LIVER CHIP:** A microfluidic liver chip was created with cell culture and flow chambers separated by a microfabricated baffle as a barrier that separates cultured hepatocytes from fluid flow to mimic the endothelial–hepatocyte interface of the liver sinusoid. The geometry of the cell culture chamber promotes linear alignment of hepatocytes in two lines, which facilitates the production of functional bile canaliculi along hepatic-cord-like structures. Reproduced from ref. 9 with permission. **KIDNEY CHIP:** A simplified kidney-on-a-chip that mimics the interface between epithelium and flowing urine was created by bonding a microfabricated PDMS channel and a PDMS well to a common semi-permeable membrane on which collecting duct epithelial cells are cultured and subjected to fluid flow. Reproduced from ref. 10 with permission. **LUNG CHIP:** A human breathing lung-on-a-chip was fabricated from PDMS that reconstitutes the structural organization, mechanical activity, and physiological functionality of the alveolar–capillary interface of the living human lung. This was accomplished by bisecting a central microfluidic channel horizontally with a porous flexible PDMS membrane, coating it with ECM, and culturing human alveolar epithelial cells on one side and pulmonary microvascular endothelial cells on the other. These tissues also were exposed to physiological mechanical forces that mimic breathing movements and blood flow by applying cyclic suction to the hollow side chambers and flowing medium through the capillary channel, respectively (also see Fig. 2). Reproduced from ref. 13 with permission. **BODY CHIP:** A microfluidic device containing multiple linked tissue types representing different organs was constructed by sealing three microfabricated silicon cell culture chambers against a plastic cover. Each cell culture chamber contains a 3D ECM gel containing living cells from a different organ. Medium was recirculated through the chambers *via* linked microfluidic channels during operation. Reproduced from ref. 35 with permission.

therapies based on magnetic field guidance of superparamagnetic submicron particles.

In contrast to these highly simplified microengineered models, chemicals, nutrients, hormones, metabolites, cytokines, and physical signals are normally transferred across interfaces between adjacent living tissues (*e.g.*, epithelium/mesenchyme, parenchymal cells/endothelium) in organs within our body. Recently microengineering approaches also have been used to recreate these key structural relationships and permit study of these processes *in vitro*. For example, a living model of the human alveolar-capillary interface was created by microfabricating a flexible PDMS device containing a central microfluidic channel and two hollow side chambers.<sup>13</sup> A thin (10  $\mu\text{m}$ ) PDMS membrane containing an ordered array of microengineered pores (10  $\mu\text{m}$  diameter with a 10  $\mu\text{m}$  spacing) coated with ECM was stretched horizontally across the center of the main microchannel (Fig. 1). Human alveolar epithelial cells were cultured on one side of the porous membrane and overlaid with air, while human lung capillary endothelial cells were cultured on the opposite side of the same membrane and overlaid with flowing medium.

A microfluidic cornea model also was developed to create a fully biological tissue–tissue interface by culturing primary rabbit corneal epithelial cells on a thin collagen gel membrane that was suspended across a PDMS microchannel.<sup>14</sup> The use of microfluidics in this system made it possible to enzymatically degrade the collagen membrane after the epithelium had formed and deposited its own basement membrane, and then to seed corneal stromal cells (keratocytes) on the basal surface of the epithelium. This effectively enabled layer-by-layer assembly of a purely cellular tissue construct with an endogenous ECM that retained physiological barrier function. On-chip permeability assays performed in this microengineered cornea model detected corneal epithelial damage induced by addition of sodium hydroxide, and hence this microengineered device could offer a low-cost alternative to animal studies for screening ocular irritants in humans.

Microengineering techniques have been used to design and fabricate novel 2D and 3D microstructures that enable the formation of *in vivo*-like tissue–tissue interfaces in the brain. For example, carefully designed asymmetric microchannels were used to reproduce complex, oriented neuronal networks.<sup>15</sup> This system consists of two cell culture chambers separated by an array of gradually narrowing funnel-shaped microchannels that serve as directionally selective filters for unidirectional axonal growth. Co-culture of different neuronal subtypes in this microdevice reproduced physiological cortico-striatal networks in which cortical afferents formed active connections to striatal neurons cultured in a separate chamber and promoted their functional and morphological differentiation. Similarly, micro-fabricated elastomeric valves were used to allow precise positioning and co-culture of hippocampal neurons and glia for several weeks.<sup>16</sup>

Microsystems engineering approaches also have been applied to investigate complex interplay between adjacent tissue types during disease development and progression. One example is a microfluidic model of breast cancer in which passive (surface tension-driven) pumping and laminar flow patterning techniques were used to form an ECM gel containing human mammary tumor epithelial cells closely juxtaposed to another gel layer

containing human mammary fibroblasts to mimic the tumor–stroma interface.<sup>17</sup> By controlling the distance between the two cell types using a blank spacer gel of varying width, this microsystem showed that paracrine signaling over long distances mediated by soluble factors may drive the invasive transition during cancer progression and that direct cell–cell contact appeared to be required for invasion to continue.

The role of tumor cell adhesion to the vascular endothelium during cancer metastasis was studied in another microfluidic model that was lined by cultured human microvascular endothelial cells and perfused with human breast cancer cells.<sup>18</sup> Studies using this model showed that stimulation of the basal surface of the endothelium with CXCL12 chemokine under physiological flow conditions leads to substantially increased adhesion of circulating breast cancer cells, suggesting the possibility of blocking cancer metastasis by targeting CXCL12 signaling.

## Recreating relevant chemical microenvironments

Biomimetic microsystems strategies have been exploited to create and engineer relevant chemical microenvironments as well. For instance, *in vivo*-like gradients of nutrients were produced in a microfluidic perfusion culture system equipped with hydrodynamic traps to maintain the morphology and function of pancreatic islets isolated from mice.<sup>19</sup> This microengineered configuration produced a two-fold increase in endothelial cell density within the cultured islets compared to classic *in vitro* cultures, which was attributed to increased diffusion of serum albumin into the center of the islets. Perfusion cultures were also applied to 3D liver culture systems to maintain viability of 3D liver tissue and liver-specific function such hepatic transport.<sup>20,21</sup> Recreation of physiologically relevant oxygen gradients in a perfused bioreactor led to regional variations in hepatocyte function along the liver sinusoids that mimic those characteristic of normal liver zonation.<sup>21,22</sup> Combination of microfluidics with paper-based 3D cell culture systems that facilitate creation and control of chemical and oxygen gradients in 3D cultures may provide yet another way in the future to generate relevant variations in these chemical parameters that are critical for organ formation and function.<sup>23</sup>

In another example of a microfluidic system, chemotaxis of cancer cells during metastasis was modeled in a multilayered microfluidic device in which cancer cells were hydrodynamically patterned in a microchannel at spatially defined positions relative to ‘source’ cells that secrete chemoattractants and ‘sink’ cells that scavenge the chemokines (Fig. 2a).<sup>24</sup> Studies in this system revealed that cancer cell migration induced by chemotactic factors depends on the location of the sink cells relative to the source cells, which determines the slope of the chemotactic gradient. By recreating a more physiological microenvironment, this microdevice enabled efficient chemotaxis under much shallower chemoattractant gradients than was previously possible in other *in vitro* systems.

Another microfluidic approach was used to study control of mesenchymal condensation that drives odontogenic differentiation during tooth organ formation in the embryo.<sup>25</sup> In this study, the microfluidic device was used to generate steep and shallow gradients of the chemoattractant (Fgf8) and chemorepellent

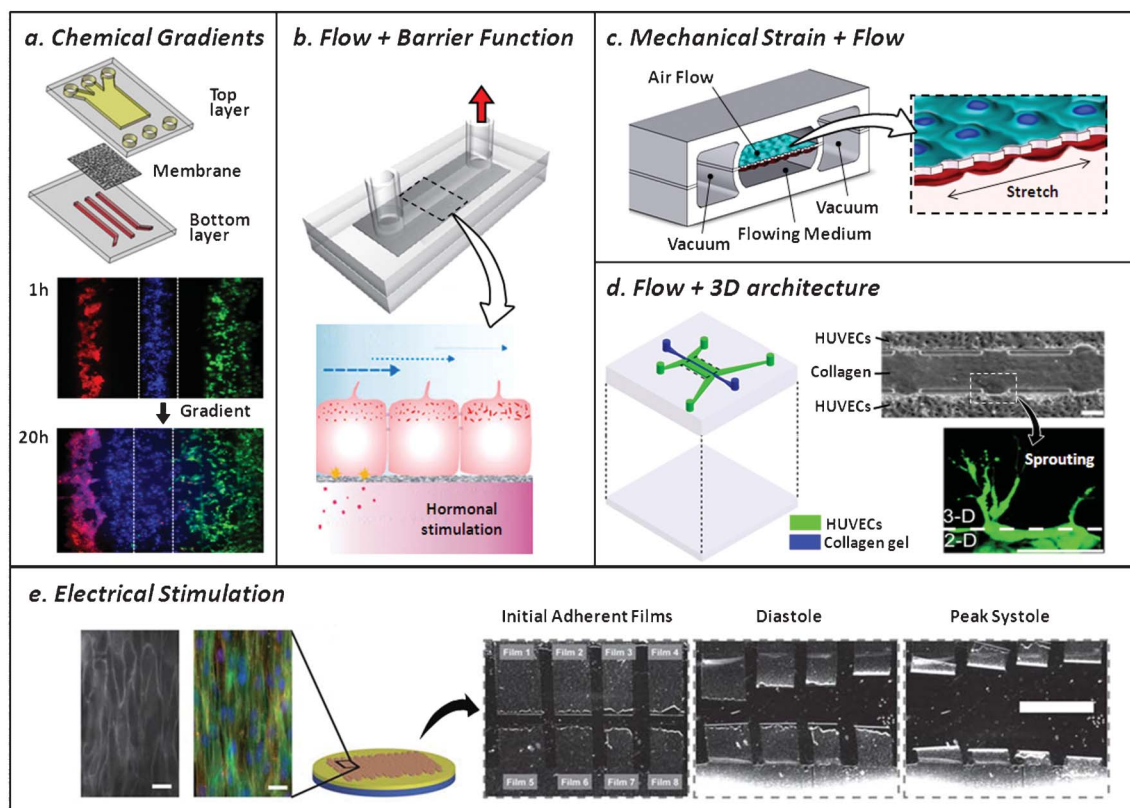


**Fig. 2 Reconstituting relevant chemical and mechanical microenvironments in microengineered organ models.** a. **CHEMICAL GRADIENTS:** An *in vitro* model of tumor-stromal interactions was engineered in a microfluidic device consisting of two PDMS microchannels separated by a semi-permeable membrane. Cancer cells were hydrodynamically patterned in the top channel at spatially defined positions relative to source and sink cells, which generated physiological chemotactic gradients that influenced cancer cell migration. Reproduced from ref. 24 with permission. b. **FLOW + BARRIER FUNCTION:** The transport barrier of the kidney was modeled by creating a microfluidic system containing a microfabricated PDMS channel separated from an underlying PDMS well by a semi-permeable membrane on which kidney epithelial cells were cultured. Application of continuous hydrodynamic flow that produces shear stresses similar to those of the kidney collecting duct result in expression of differentiated epithelial cell functions. Reproduced from ref. 26 with permission. c. **MECHANICAL STRAIN + FLOW:** The alveolar-capillary interface that was recreated in the breathing lung-on-a-chip shown in Fig. 1 using living human pulmonary epithelial and endothelial cells cultured on opposite sides of a thin, ECM-coated, porous, flexible membrane was subjected to cyclic mechanical strain and fluidic dynamics that mimic breathing motions and physiological blood flow by respectively applying intermittent suction to two vacuum chambers on either side of the central cell-containing microchannel, and flowing medium through the microvascular channel. Reproduced from ref. 13 with permission. d. **FLOW + 3D ARCHITECTURE:** A microfluidic model of sprouting angiogenesis in which human umbilical vein endothelial cells (HUVECs; green) are cultured in two parallel microchannels separated by a 3D collagen gel (blue). Endothelial sprouting in this system was optically monitored while applying fluid shear stress and/or inducing interstitial flow through the 3D collagen gel (bar, 100  $\mu$ m). Reproduced from ref. 28 with permission. e. **ELECTRICAL STIMULATION:** A microengineered heart model cultures neonatal rat ventricular cardiomyocytes on thin PDMS films coated with micropatterned ECM to form anisotropic contractile cell monolayers, and then applies electrical stimulation (bar, 20  $\mu$ m). Laser cutting of three edges of the film creates cantilevered films with adherent cardiomyocyte cell layers that remain attached to the substrate along one edge. An external field stimulator is used to simultaneously pace multiple cardiomyocyte thin films at 2 Hz with 5–20 V, and contractile stresses are measured by quantitating changes in curvature of the PDMS cantilevers (right; bar, 5 mm). Reproduced from ref. 30 with permission.

(Sema3f), respectively, within a single microchannel. When embryonic mesenchymal cells were cultured in this device, cells moved over long distances up the Fgf8 gradient, while being repulsed locally by Sema3f; this resulted in compaction of cells within a tight ball that recapitulated the mesenchymal condensation that drives embryonic tooth organ formation *in vivo*.

### Recapitulating the physical microenvironment

While microfluidics has been used for many years to generate defined chemical gradients, they also recently have been leveraged to mimic the physical microenvironment that cells

and tissues experience in living organs. Mechanical forces have long been recognized as a key determinant of differentiated functions, and the effects of fluid shear stress in the kidney-on-a-chip<sup>10</sup> described above (Fig. 2b) is a salient example. But in the kidney model, not only did application of normal levels of fluid shear stress induce morphological polarization, it also induced the epithelium to display transport functions normally only seen in whole living kidney, including increased transport of water and sodium in response to hormonal stimulation with vasopressin and aldosterone. Application of physiological levels of fluid shear stress to the apical surface of these cells also triggered reorganization of the actin cytoskeleton and trafficking of water

transport protein (aquaporin-2), which may be useful for drug screening and renal physiology studies.<sup>26</sup>

Other organs experience multiple types of mechanical cues simultaneously. For example, alveolar epithelial cells in the air sacs of the lung experience cyclic stretching deformations and air flow due to breathing motions while underlying pulmonary capillary endothelial cells on the opposite side of the same basement membrane ECM experience fluid shear stresses due to blood flow, as well as cyclic strain. The lung-on-a-chip described above was designed so that in addition to recreating the alveolar-capillary interface (Fig. 1), these cultured human cells also could be exposed to physiologically relevant mechanical cues (Fig. 2c).<sup>13</sup> This was accomplished by flowing culture medium continuously through the microvascular channel to apply a physiological level of fluid shear stress (1 dyne/cm<sup>2</sup>), and primary human neutrophils were sometimes included to study immune responses. Application of intermittent suction to the side chambers resulted in the application of levels of cyclic strain (10% strain; 0.2 Hz) to the flexible PDMS membrane and adherent pulmonary endothelium and epithelium that precisely mimicked those experienced by cells at the alveolar-capillary interface *in vivo*.

Application of physiological cyclic strain and flow of both air and medium through the air and blood channels of the lung-on-a-chip not only induced tissue differentiation and formation of tissue–tissue interfaces, it also promoted expression of complex functionalities never before observed in cell culture systems.<sup>13</sup> For example, this breathing lung-on-a-chip replicated complex organ-level responses to bacteria and inflammatory cytokines by inducing expression of intercellular adhesion molecule-1 (ICAM-1) on the microvascular endothelium surface, adhesion of circulating human neutrophils, their transmigration across the capillary-alveolar interface, and phagocytosis of the infectious pathogens, which can be visualized using real-time, high-resolution microscopy. Nanotoxicology studies using this system also revealed that physiological cyclic strain exacerbates acute toxicity of silica nanoparticles delivered to the alveolar space by promoting rapid release of toxic reactive oxygen species by alveolar epithelial cells, upregulating endothelial ICAM-1 expression and stimulating neutrophil adhesion. Cyclic mechanical strain that mimics normal breathing motions also increased nanoparticle transport across the lung tissue layers into the underlying microvascular channel and enhanced extrapulmonary absorption of these particles. Importantly, the effects of physiological breathing on nanoparticle absorption across the alveolar-capillary barrier predicted by the lung-on-a-chip microdevice were confirmed in a mouse *ex vivo* lung ventilation–perfusion model.<sup>13</sup> Thus, this simple device not only mimicked organ-level functions, but it led to the discovery of previously unknown physiological organ-level responses in a human relevant model.

A similar approach was recently used to create a human gut-on-a-chip microdevice that exerts cyclic mechanical strain and fluid flow on cultured human Caco-2 intestinal epithelial cells to mimic physiological peristaltic motions and fluid flow of living intestine.<sup>27</sup> Culture of Caco-2 cells under these physiologically relevant conditions results in spontaneous formation of 3D villous-like undulated structures lined by polarized columnar epithelial cells that exhibit normal intestinal barrier functions as

measured by quantifying transepithelial electrical resistance and paracellular permeability. Moreover, this microfluidic human intestinal model also permits long-term co-culture of normal gut microflora (*Lactobacillus* sp.) with host epithelial cells, which actually enhances intestinal barrier functions.

Effects of physiological fluid forces combined with 3D tissue microarchitecture were also examined in a microengineered model of vascular sprouting that consists of two parallel cell culture channels lined with confluent monolayers of human umbilical vein endothelial cells (HUVECs) separated by a microchannel filled with a collagen gel (Fig. 2d).<sup>28</sup> This microsystem contains regularly spaced 100 µm-wide apertures along the length of the central microchannel that permit contact between the endothelial cells and the collagen matrix, and enable direct visualization and quantitative analysis of vascular sprouting. By precisely controlling fluid convection in the vascular compartment and inter-vessel matrix, this model revealed that physiological levels of fluid shear stress generated by blood flow attenuate endothelial cell sprouting, whereas fluid forces induced by interstitial flow enhances sprout formation.

Other relevant recent developments include the microengineering of disease models that recapitulate pathological physical microenvironments, such the mechanical distortion of cells that produces ventilator-induced lung injury.<sup>29</sup> In one microdevice, human alveolar epithelial cells were cultured on a thin flexible PDMS membrane that overlays an actuation channel filled with fluid. When fluid was withdrawn from the actuation channel, the intervening PDMS membrane deformed, thereby stretching the cells adherent to the opposite side of the membrane and causing propagation of a fluid meniscus in the cell culture chamber. Studies using this ‘Alveolus-on-a-Chip’ demonstrated that a combination of fluid and solid mechanical stresses elicits significantly more injurious cellular responses than either alone, and that this detrimental effect can be reduced by addition of surfactant.

In addition to mechanical cues, microsystems approaches have been developed to apply relevant electrical stimulation to cultured cells, and to study specific cellular responses. For example, neonatal rat ventricular cardiomyocytes have been cultured on thin flexible PDMS films that were micropatterned with ECM proteins to promote spatially ordered, two-dimensional myogenesis, and then to apply electrical stimulation to these formed contractile tissues (Fig. 2e).<sup>30</sup> These heart tissue constructs are electrically functional and mechanically active: they generate contractile stresses comparable to those produced by whole papillary muscle. This heart-on-a-chip system enables direct monitoring and measurement of heart muscle contractility combined with quantification of action potential propagation and cytoskeletal architecture in multiple tissues. Thus, this device provides a robust platform to measure effects on heart cell contractile function *in vitro* in response to pharmacological stimulation to determine drug efficacy and cardiotoxicity.

## Organ–organ integration

Ultimately, human physiology is a manifestation of interactions between different organs. Microfluidic approaches have been leveraged to culture frozen thick sections of whole living organs (*e.g.*, brain, liver) as one approach to mimic organ-level

functions, and to link organs microfluidically for physiological studies.<sup>31–33</sup> Responses to electrical stimuli, drug metabolism, and absorptive events have all been monitored using this approach. However, it is not possible to recreate the normal mechanical and chemical microenvironment in these studies, and the viability of these specimens is severely limited (< 5 days),<sup>32</sup> which likely will restrict their use for drug testing and toxicological studies in the future.

As an alternative approach, researchers have microfabricated 'Body-on-a-Chip' devices that contain multiple microchambers, each of which contains a different type of cultured cell (e.g., liver epithelium vs. brain neurons) connected by a network of microfluidic conduits that permit recirculation and exchange of metabolites in a physiologically-relevant manner.<sup>34</sup> Early attempts at this approach were limited by lack of optimal tissue-specific differentiation or organ-relevant microenvironments. More recently a microfluidic platform was developed that incorporates 3D hydrogel cultures of multiple cell types to address the limitations of previous 2D cell culture analogs and to provide more physiological culture conditions.<sup>35</sup> This system was created by injecting human cell-containing Matrigel and alginate solutions into microchambers and subsequently inducing their in-channel polymerization to form three interconnected compartments with 3D tissue constructs that represent the cells of the liver, bone marrow, and a colon tumor (Fig. 1). When an anti-cancer drug (Tegafur) was perfused through this microfluidic system, it was possible to detect effects on efficacy, hepatotoxicity, and hematological toxicity that were not observed in conventional tissue culture models, and that more closely mimicked results obtained *in vivo*. The design of this microfluidic device also was recently modified to eliminate the need for external pumps and enable more facile operation using gravity-induced flow.<sup>36</sup> This approach was further strengthened by development of a mathematical model of the pharmacokinetic and pharmacodynamic profiles of the anti-cancer drug, which can be used to predict experimental results obtained from the microfluidic system. Similar strategies were adapted to examine intestinal absorption, hepatic metabolism, and bioactivity of breast cancer drugs in a multilayered microfluidic system that supports cultures of human intestinal epithelial cells, hepatocytes, and breast cancer cells in separate microfabricated chambers.<sup>37</sup> One can envision that in future multi-organ physiological systems, it might be possible to use the heart-on-a-chip to actively and naturally pump fluid through the whole system, while using the lung chip to oxygenate, liver chip to metabolize, kidney chip to cleanse the circulating blood substitute, and so on. However, in the near-term, it is likely that external pumps will be used to drive fluid flow and to control variations in fluid flow and dynamics that are required to maintain viability of many different organ chips linked within a single, integrated, chip-based, multi-organ physiological test system.

## Future opportunities and challenges

These organ-on-chip microsystems could have a significant impact on the future of the pharmaceutical industry by streamlining the drug discovery process and replacing animal models for efficacy and toxicity testing. The pharmaceutical and

biotechnology industries are currently facing rising research and development costs, dwindling pipelines, and pressure from governments to reduce health care costs.<sup>38</sup> They are further plagued by costly high attrition rates at later, and more costly, clinical stages of the drug development, with many drugs even undergoing withdrawal after reaching the market. Animal testing that is required for determination of therapeutic efficacy and safety is currently one of the major bottlenecks because it is costly and time-consuming; it also requires large amounts of compounds that are often not available at earlier stages in the drug discovery pipeline. Most worrisome is that traditional animal testing approaches often fail to predict human toxicity and efficacy,<sup>39</sup> and many now question the ethics of sacrificing animals if they cannot reliably predict clinical outcomes. For example, cardiac toxicity by non-cardiac drugs is the most common cause of drug development delays, FDA failure, and market withdrawal, yet it is difficult to predict human cardiotoxicity using conventional *in vitro* heart cell-based systems or animal models.<sup>40,41</sup> There is therefore intense pressure economically, ethically and scientifically to find ways in which to improve the success of the drug development process, and identify suitable alternatives that can circumvent the need for animal studies.

The technologies reviewed have the potential to be more predictive human relevant models for toxicity and efficacy testing, and to provide insight into mechanisms of action at the tissue and organ levels. *In vitro* human cell-based models are already being successfully applied to predict some aspects of absorption, distribution, metabolism and excretion (ADME) of many drug candidates, such as the use of primary human hepatocytes for prediction of drug-drug interactions in humans.<sup>42</sup> The successful implementation of these and other tools for ADME predictions has led to a marked decrease in clinical failure rates due to poor pharmacokinetic properties or bioavailability; however, this approach has not improved failure rates due to lack of efficacy or toxicity.<sup>42</sup> The organs-on-chips microsystems described here offer an alternative approach by creating human-relevant disease models (e.g., pulmonary edema on a chip, Crohn's disease on a chip, etc.) for efficacy testing. Such models could have an impact on various stages of the drug development process, including identification and prioritization of lead compounds, as well as target validation (e.g., by providing improved mechanistic understanding in a human relevant system). In addition, collections of different organ chips integrated *via* microfluidic linkages (Fig. 3) could provide a way to model physiological interplay between different organs (e.g., linking the gut and liver to gain better insight into the interplay between transporters and drug-metabolizing enzymes) that is critical for the determination of drug ADME properties. This is not currently possible with conventional *in vitro* cell culture models, and differences in transcriptional regulation of these enzymes and transporters precludes extrapolation from animal data.

Organ-on-chip technologies also might have a significant impact on environmental toxicology and industrial safety applications. More predictive and reproducible *in vitro* assays using organs-on-chips containing human cells could expand the number of environmental toxicants that are evaluated, as well as reduce the time, cost, and number of animals involved in safety

testing. If successful, this new screening technology would transform the field of environmental toxicity evaluation by shortening testing times, decreasing costs, and informing regulatory decision-making agencies (e.g., EPA) earlier in the process with more predictive human relevant data. A similar approach could be used to determine biopharmaceutical and chemical safety in the manufacturing pipeline, or be used for water testing.

Another driving force behind the need for organ-on-chip alternatives is the change in the regulatory climate. The 2007 European Union regulatory policy on chemicals and their safe use – REACH (Registration, Evaluation, Authorization and Restriction of Chemical substances) – aims to increase information regarding the safety and hazards posed by chemicals for human health and the environment.<sup>43</sup> Implementation of the REACH regulation requires that the number of animal tests be minimized, and that *in vivo* testing should be avoided and only carried out as a last resort. This is further fueling the need to find alternative testing models to animals.

One of the key elements required for implementing organ-on-chip models in pharmaceutical screening, environmental safety testing, or regulatory studies is to carry out *in vitro* studies using these microsystems with compounds and drugs that have already been extensively characterized in humans. In this way, the safety and toxicity response measured *in vitro* can be validated in terms of their ability to predict human responses *in vivo*. Thus, extensive additional supporting data will need to be generated, likely in close collaboration with the pharmaceutical industry and regulatory authorities, in order to build the confidence all parties will require before these novel organomimetic systems could be accepted as true alternatives to existing animal models.

Thus, it is likely that development of clinically relevant endpoints and the ability to extrapolate data obtained *in vitro* to results in humans will be key determinants of the future success of organ-on-chip technologies. In addition to ensuring relevant endpoints are measured and validated, investigators in this field will need to develop pharmacokinetic and pharmacodynamic (PK/PD) models to extrapolate clinically relevant behavior profiles from *in vitro* data. These PK/PD models will need to include mathematical models of individual organ chips to account for device geometry, cell and tissue properties, fluid and solid mechanics, and perfusion, as well as cell transport and metabolic activities. They also must be able to consider the contributions of convection, diffusion, and chemical reactions, as well as transport of free/bound drug, adsorption on surfaces (e.g. air/mucus), transport across barriers, ECM adhesion, cell membrane binding, and intracellular distribution and processing, in order to establish relevant *in vitro*–*in vivo* correlations.

Despite the many desirable properties of PDMS for the development of microfluidic systems (e.g., simple fabrication, high biocompatibility, optical transparency and flexibility), the use of PDMS could raise problems for drug discovery applications because it can absorb small hydrophobic molecules, including certain drugs, fluorescent dyes, or cell signaling molecules.<sup>44</sup> This might result in reduction of effective drug concentrations, cross-contamination, lower detection sensitivities, and higher background fluorescence. In addition, PDMS is not optimal for scaling up manufacturing of microfluidic devices, and residual uncrosslinked oligomers may leach from PDMS

and interact with cells and culture medium.<sup>45</sup> Therefore, it will be essential to identify alternative materials with similar properties, particularly for drug discovery applications, in the future.

Another challenge for these technologies is to identify supplies of optimal human cells for each organ application. In cases where there are no appropriate cell lines or primary cells are not practical options, stem-cell derived inducible pluripotent (iPS) cells or embryonic stem (ES) cells may provide alternative solution in the future. However, while progress is being made in this rapidly developing field, much work is still required to drive most existing stem cells from their characteristic neonate-like state to a more mature adult differentiated phenotype. Again, the ability of more sophisticated organs-on-chips to provide organ-relevant chemical and mechanical microenvironments could help overcome this limitation by promoting stem cell differentiation *in situ* within the devices.

Despite the considerable advances in the development of the microengineered tissue and organ models described here, the challenge of integrating multiple organ chips in a physiologically relevant way to model whole human physiology remains a huge challenge. “Plug-and-play” instrumentation will need to be developed that not only supports fluidic connections of multiple chips and integration in a physiologically relevant manner, but also provides appropriate systems for real-time control, monitoring, visualization and feedback, as well as sample collection and analysis (Fig. 3). Successful commercial adoption of organ-on-chip technologies will be highly dependent on the instrumentation that is developed. The ultimate instrument also should provide a user interface that is easy to use, enables automation,

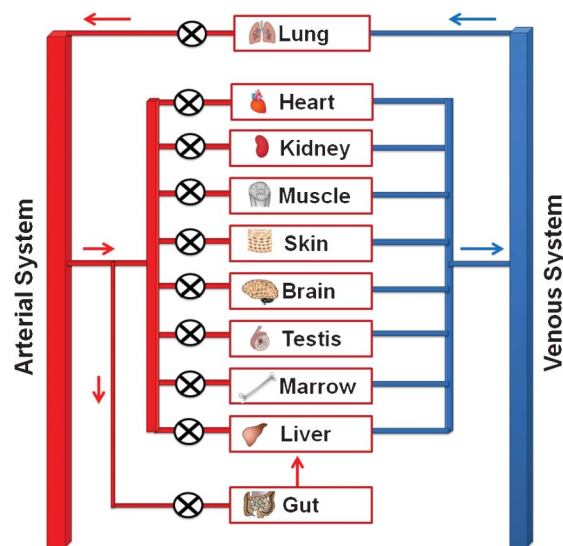


**Fig. 3 Schematic of an integrated multi-organ systems instrument.** Ten different organ chips can be integrated within a single device through linkages to a microfluidic circulatory system. A blood substitute is perfused through the entire system, and in-line valves (white X-circles) can be selectively opened or closed to regulate flows and pressures to each organ, and to couple flows between different organ combinations. Environmental monitors and pressure regulators and pumps need also to be included in the system to ensure continuous flow and maintenance of physiologically relevant conditions. Automated integration of multiple organ chips in a physiologically relevant manner will increase utility and impact of the organ-on-chip technology.

and ensures reproducibility of the data. These types of sophisticated technologies are likely not appropriate for high-throughput drug or chemical screening, rather they offer the ability to produce high quality/high content data at key stages of the drug discovery process, which could enable key decision making and replace non-predictive animal models. Another advantage of the organ-on-chip technologies lies in the fact that they offer the potential to develop complex models of human disease to study mechanism of action, identify novel disease targets, and determine drug efficacy in human-relevant systems. Thus, the capacity of instruments developed for analysis of future multi-organ physiology using organs-on-chips does not need to be high throughput; however, it must provide an ultra-high level of sensitivity, reproducibility, and robustness.

Successful integration of different organs-on-chips also will require identification of a common medium or 'universal blood substitute' that can be perfused through the entire system to maintain viability and differentiated functions of multiple relevant linked organ chips (Fig. 3). The blood substitute will have to provide all of the chemicals, nutrients, cytokines, and trophic factors required by individual organs, as well as physiologically relevant 'vascular connections' and blood composition (e.g., plasma protein binding) that effectively mimic PK/PD profiles observed in humans. Moreover, the control system of the instrument will have to incorporate pumps, valves, and pressure regulators to ensure physiological flow of the blood substitute between (and around) organs when different organ combinations are selected for analysis (Fig. 3). For example, more sophisticated versions that permit temporary linkage of specific subsets of organs (e.g., liver/kidney/heart vs. liver/intestine/brain) for dynamic analysis (e.g., drug metabolism/clearance), while also ensuring continuous perfusion to maintain viability of all organs, will likely need to be developed over time. In future multi-organ systems, it will be equally important to maintain organ-relevant fluid pressures and flows that vary between different organs in our bodies, which will likely be required for optimal tissue viability and function.

## Conclusion

Microengineered organ-on-chip technologies that combine defined 3D microsystem architectures, cultured living cells, and microfluidic linkages are relatively new and still require further validation and characterization. However, their potential to predict response in animals and humans is tremendous, and if successful, this disruptive technology could have profound effects on drug development as well as chemical and nanotoxicology testing. Future success will require academic investigators to collaborate with industry and regulatory agencies to develop appropriate biomarkers, clinically-relevant endpoints, and linked computational PK/PD models that will be necessary for system validation and extrapolation to humans. It also will be necessary to develop new microsystems designs and identify new materials and cell sources that will support scale-up of organ-on-chip manufacturing to support end user demand. Automated instruments will need to be developed with user-friendly interfaces that provide systems-level microenvironmental control, monitoring and real-time analysis of multiple, linked, living, human organs-on-chips to ensure adoption of the

techniques by industry. As a field, we have to move beyond microsystems prototyping to confront these challenges that are necessary to translate organs-on-chips from laboratory curiosities to robust animal replacements that can be used in commercial laboratories in the pharmaceutical, biotechnology, chemistry and environmental safety industries.

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