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Microfluidic organs-on-chips

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An organ-on-a-chip is a microfluidic cell culture device created with microchip manufacturing methods that contains continuously perfused chambers inhabited by living cells arranged to simulate tissue- and organ-level physiology. By recapitulating the multicellular architectures, tissue-tissue interfaces, physicochemical microenvironments and vascular perfusion of the body, these devices produce levels of tissue and organ functionality not possible with conventional 2D or 3D culture systems. They also enable high-resolution, real-time imaging and in vitro analysis of biochemical, genetic and metabolic activities of living cells in a functional tissue and organ context. This technology has great potential to advance the study of tissue development, organ physiology and disease etiology. In the context of drug discovery and development, it should be especially valuable for the study of molecular mechanisms of action, prioritization of lead candidates, toxicity testing and biomarker identification.

Conventional two-dimensional (2D) cell cultures were developed almost a century ago[1]. Despite their demonstrated value in biomedical research, they cannot support the tissue-specific, differentiated functions of many cell types or accurately predict *in vivo* tissue functions and drug activities[2]. These limitations have led to increased interest in more complex 2D models, such as those that incorporate multiple cell types or involve cell patterning, and in three-dimensional (3D) models, which better represent the spatial and chemical complexity of living tissues. 3D cell cultures, developed over 50 years ago[3], usually rely on hydrogels, composed of either natural extracellular matrix (ECM) molecules or synthetic polymers, which induce cells to polarize and to interact with neighboring cells. They can take many forms, including cells randomly interspersed in ECM or clustered in self-assembling cellular microstructures known as organoids. 3D models have been very useful for studying the molecular basis of tissue function and better capture signaling pathways and drug responsiveness in some disease states compared with 2D models[4–7]. Nonetheless, they also have limitations. For example, organoids are highly variable in size and shape, and it is difficult to maintain cells in consistent positions in these structures for extended analysis. Another drawback of 3D models is that functional analysis of entrapped cells—for example, to quantify transcellular transport, absorption or secretion—is often hampered by the difficulty of sampling luminal contents, and it is difficult to harvest cellular components for biochemical and genetic analysis. In addition, many systems lack multiscale architecture and tissue-tissue interfaces, such as the interface between vascular endothelium and surrounding connective tissue and parenchymal cells, which are crucial to the function of nearly all organs. Furthermore, cells are usually not exposed to normal mechanical cues, including fluid shear stress, tension and compression, which influence organ development and function in health and disease[8,9]. The absence of fluid flow also precludes the study of how cultured cells interact with circulating blood and immune cells.

Microfluidic organs-on-chips offer the possibility of overcoming all of these limitations. In this Perspective, we discuss the value of this new approach to scientists in basic and applied research. We also describe the technical challenges that must be overcome to develop organs-on-chips into robust, predictive models of human physiology and disease, and into tools for drug discovery and development.

What are organs-on-chips?

Microfluidic culture devices. Organs-on-chips are microfluidic devices for culturing living cells in continuously perfused, micrometer-sized chambers in order to model physiological functions of tissues and organs. The goal is not to build a whole living organ but rather to synthesize minimal functional units that recapitulate tissue- and organ-level functions. The simplest system is a single, perfused microfluidic chamber containing one kind of cultured cell (e.g., hepatocytes or kidney tubular epithelial cells) that exhibits functions of one tissue type. In more complex designs, two or more microchannels are connected by porous membranes, lined on opposite sides by different cell types, to recreate interfaces between different tissues (e.g., lung alveolar-capillary interface or blood-brain barrier). These systems can incorporate physical forces, including physiologically relevant levels of fluid shear stress, cyclic strain and mechanical compression, and permit analysis of organ-specific responses, including recruitment of circulating immune cells, in reaction to drugs, toxins or other environmental perturbations. Similar analyses can be conducted with chips lined by cells from different organs that are linked fluidically, either directly from one interstitial tissue compartment to another, or potentially through a second channel lined with vascular endothelium, to mimic physiological interactions between different organs or to study drug distribution *in vitro*.

The word 'chip' in organ-on-a-chip stems from the original fabrication method, a modified form of photolithographic etching used to

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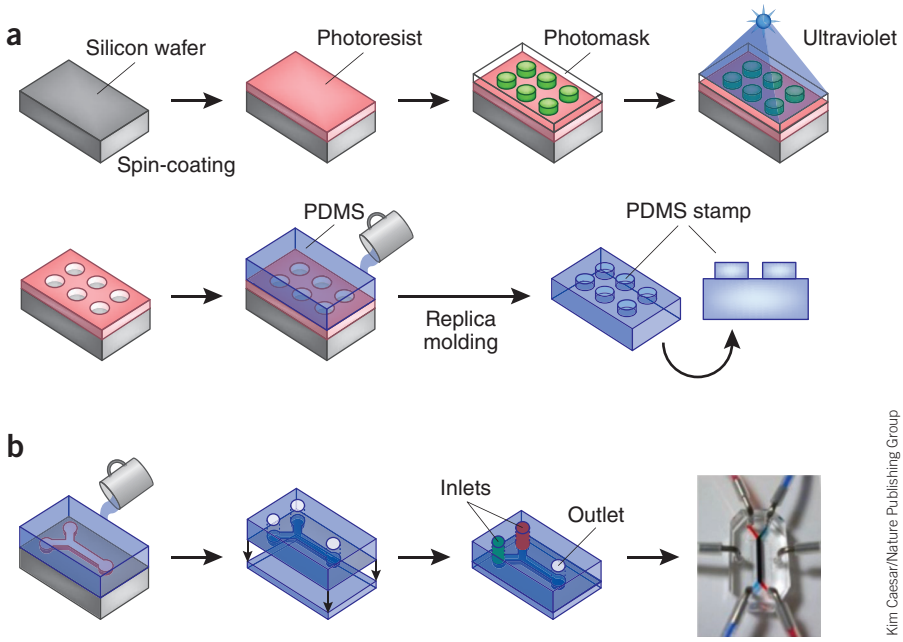

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Figure 1 Fabrication methods for microfluidic chips. (**a**) Replica molding[10] creates stamps with shapes complementary to patterns etched in silicon chips by photolithography. A thin uniform film of a photosensitive material (photoresist) is spin-coated on a silicon chip, which is then overlaid with a photomask (e.g., a transparent glass plate patterned with opaque chrome layers) bearing a microscale pattern generated with computer-assisted design software. The photomask protects some regions of the photoresist and exposes others during exposure to high-intensity ultraviolet (UV) light. The UV-exposed material dissolves in a developer solution, leaving the microscale pattern etched into the photoresist. Elastomeric stamps with a surface topography complementary to the etched surface are created by a replica-molding technique in which liquid prepolymer of PDMS is cast on top of the etched photoresist pattern, polymerized and peeled off. The PDMS stamp can be used for microcontact printing of ECM molecules on any substrate, including those within microfluidic devices (not shown). (**b**) A single-channel microfluidic device is fabricated by making a PDMS stamp with two inlets, a single main channel and one outlet and conformally sealing it to a flat glass substrate. A photograph of a two-chamber microfluidic culture device, with red and blue dye are perfused through upper and lower channels, is shown at the right. The clear side channels are used to apply cyclic suction to rhythmically distort the flexible central membrane and adherent cells.

manufacture computer microchips, which allows control of surface feature shapes and sizes on the same scale (nm to μm) that living cells sense and respond to in their natural tissue milieu. Microfluidic culture systems are often made by 'soft lithography', a means of replicating patterns etched into silicon chips in more biocompatible and flexible materials[10]. This is done by pouring a liquid polymer, such as poly-dimethylsiloxane (PDMS), on an etched silicon substrate and allowing it to polymerize into an optically clear, rubber-like material, essentially creating a rubber stamp (**Fig. 1a**). Soft lithography was first used to pattern microscale adhesive islands made of ECM molecules as a way to specify the shape, position and function of cells cultured on silicon chips[11], and later on conventional culture substrates[12,13] (work by D.E.I. and colleagues). Subsequently, this approach was modified by inverting the PDMS mold and conformally sealing it to a flat smooth substrate, such as glass, to create open cavities in the form of small (cross section < 1 mm × 1 mm), linear, hollow chambers, or 'microfluidic channels,' with openings at both ends of the polymer block for perfusion of fluids (**Fig. 1b**). Miniaturized perfusion bioreactors for culturing cells were made by coating the surface of the central channel with ECM molecules, flowing cells into the channel so that they adhere to the ECM substrate, and then perfusing the channel continuously with culture medium[14,15] (**Fig. 2a**). A key feature of PDMS culture systems is that they are optically clear (**Fig. 1b**), which allows real-time, high-resolution optical imaging of cellular responses to environmental cues. Similar devices were later fabricated out of various materials (e.g., silicon, plastic, glass, silk) using micromolding, microetching, laser etching, injection molding, photopolymerization, solid object printing and other microscale manufacturing approaches.

Control of system parameters. Microfluidic chips provide control over many system parameters that are not easily controlled in 3D static cultures or bioreactors, facilitating study of a broad array of physiological phenomena. Because these devices are fully microengineered, they can be integrated with microsensors that report on the cultured cells or microenvironmental conditions, which is usually not feasible in self-organized 3D cultures. Microsensors incorporated in chips have been used for analysis of tissue barrier integrity[16], cell migration[17] and fluid pressure[18]. In the future, it may be possible to detect a range of other chemical and culture conditions (glucose, lactate, oxygen, pH)[19].

Control of fluid flow in chips has proved enormously useful. For example, because viscous forces dominate over inertial ones at small length scales, the flow is laminar if the diameter of the 'microfluidic' channel is less than about one millimeter. This allows the generation of physical and chemical gradients, which have been exploited for noninvasive study of directional cell migration[20–22], cardiac tissue formation[23], nerve axon outgrowth[24], and graded metabolic[25], differentiation[26] and neurotoxin[27] responses, as well as analysis of subcellular structure[20] and cell-cell junctional integrity[28]. Fluid shear stresses can be controlled independently of physical and chemical gradients by altering flow rates or channel dimensions[29,30], and by separating cells from the flow path using a nanoporous membrane[29] or microengineered posts that restrict cell passage[31]. Fluid-mechanical computational models can be applied to optimize microchannel geometry and enhance oxygen and nutrient delivery, thereby increasing cell survival and function[29].

Control of cell patterning is another advantage of the chip format. Different cell types can be plated in distinct patterns or in direct juxtaposition on the same planar substrate in the microchannel (**Fig. 2b**) by several methods: using laminar streams to plate cells or ECM proteins[20], designing complex microchannel paths that intermittently contact the adhesive substrate[32], positioning microposts between neighboring cell types[31] or microprinting ECM in distinct positions within the channels[33–35]. The substrate can even be shaped by micromolding techniques into organ-like forms, such as the villus shape of the intestine[36]. More recent chip designs have incorporated cells embedded in 3D ECM gels[37–41] (**Fig. 3**) and multicellular constructs created by tissue engineering[35,41–43] (**Figs. 2c** and **Fig. 4a**).

The ability to integrate porous substrates to separate two parallel microchannels has enabled analysis of tissue barrier functions and transcellular transport, absorption and secretion[16,44–49] (**Fig. 4b,c**). By culturing two different cell types on opposite sides of the substrate, one can create tissue-tissue interfaces that mimic the interactions of vascular

endothelium and parenchymal tissues that define nearly all organs[44,45,47] (**Fig. 4d**).

Chips also allow the complex mechanical microenvironment of living tissues to be recapitulated *in vitro*. Cyclic mechanical strain can be produced using flexible side chambers and applying cyclic suction that rhythmically stretches and relaxes the lateral wall and attached central membrane[44,45,48,49] (**Fig. 4c**,**d**). Cells in tissues adhered to a flexible membrane can be exposed simultaneously to cyclic mechanical deformation and fluid shear stresses, similar to what most cells experience in living organs during processes such as breathing, peristalsis and cardiovascular cycling[35,44,45,48]. Tissues that normally respond to compressive forces can be actively compressed by increasing pressure in an air chamber separated from the culture chamber by a membrane[50]. Electrical fields can also be applied; these have been used to pace contractile cells[35] and to stimulate wound healing[51] on chips.

Although most studies with organs-on-chips have been carried out on established cell lines or primary cells, in principle the technology is suited to any cell type amenable to culture, including plant and insect cells (e.g., to identify pesticides or defoliants that are not toxic to humans). Chips have been applied to investigate stem cells (embryonic stem cells[52,53], induced pluripotent stem (iPS) cells[54], mesenchymal stem cells[54] and neural stem[55,56] cells) differentiated to specific lineages, as well as the differentiation process itself[57,58]. The use of stem cells, and particularly iPS cells, is exciting because of the potential to model diseased organs with patient- and disease-specific cells. Current directed-differentiation protocols generally produce immature cells, such as immature cardiomyocytes, hepatocytes and endothelial cells[53,59,60], but the problem of maturation is being addressed by multiple approaches[61,62]. Culture of stem cells in lineage-specific physical microenvironments on chips may also be beneficial in promoting differentiation. These lines of research may eventually lead to personalized 'humans-on-chips' in which all the organ chips are derived from a single patient.

Can organs-on-chips mimic organ-level functions and disease?

Organs-on-chips have great potential for the investigation of basic mechanisms of organ physiology and disease. They are particularly well-suited to the study of biological phenomena that depend on tissue microarchitecture and perfusion, and that involve relatively acute (<1 month duration) pathophysiological processes. Researchers have fabricated chips for the study of the liver[29,31,33,43,63–70], kidney[46,71–73], intestine[36,48,49,74], lung[44,45,63,75–77], heart[35,40,78–80], smooth and striated muscle[81], fat[43,63], bone[50,82,83], marrow[84], cornea[85], skin[86], blood vessels[18,44,45,47,87,88], nerve[24,41,89–91] and blood-brain barrier[30,47,92–94], among others, over the past decade. Many of these devices cannot be considered models of organs because only one cell type was cultured in one microchannel. However, they revealed that application of fluid flow and shear stress alone has potent effects on cell form and function. For example, a mixed population of primary hepatocytes in a microfluidic chamber formed two compartments of periportal-like and perivenous-like cells after 24 h under dynamic flow[67] (work by S.N.B. and colleagues). The depletion of soluble species caused by flow produced gradients of both dissolved oxygen and soluble growth factors, and oxygen tension was shown to be the major determinant of the 'zonation' response[67].

Improved function through microengineering. Cell-cell interactions are crucial for maintaining tissue structure and function, and many cells respond to both homotypic and heterotypic interactions. A liver-on-a-chip was engineered to mimic heterotypic interactions by separating primary human hepatocytes, cultured at a high homotypic cell density in a low shear stress and diffusion-dominated *in vivo*-like environment, from the active flow channel by very small (1 × 2 × 30 μm)

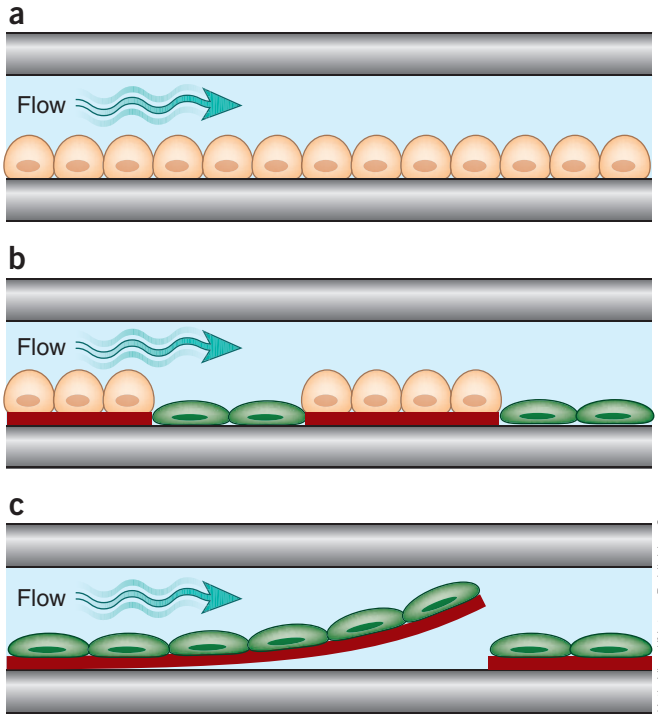

Figure 2 Examples of increasingly complex single-channel, organ-on-chip designs. (**a**) Cells of a single type are cultured as a monolayer on a planar rigid (e.g., glass) or flexible (e.g., PDMS) substrate on one side of a microfluidic channel through which medium is perfused. (**b**) Cells of two types are cultured in direct juxtaposition by micropatterning ECM adhesive islands within the microfluidic chamber that preferentially support one cell population (e.g., hepatocyte). These cells are delivered first, and the empty spaces are then filled with the second cell population (e.g., fibroblast). (**c**) Cells in a tissue construct engineered with ECM are cultured in a microfluidic channel. In this example, microcontact printing of ECM in a linear pattern on a thin PDMS layer coated over the substrate is used to orient muscle cells to create an anisotropic muscle tissue layer. When parts of the PDMS film are released from the substrate, they bend up when the cells contract, allowing measurement of cell contraction forces under flow[35].

microchannels designed to resemble the natural endothelial barrier of the liver sinusoid[31]. The device maintained the metabolic activity of the hepatocytes for over 7 d and permitted analysis of the hepatoxicity of the drug diclofenac (Voltaren), which depends on its chemical modification by functional liver cells. Heterotypic cell-cell interactions stabilize human hepatocyte function and improve the predictivity of drug metabolism and toxicity assays, even in the absence of flow[95,96], but flow-based chips enable dynamic monitoring of metabolite production[68–70].

Dynamic variation of oxygen tension has been used in organs-on-chips to invoke disease states, such as heart ischemia[40] or vaso-occlusion in sickle-cell disease due to polymerization of hemoglobin S in deoxygenated erythrocytes (work by S.N.B. and colleagues)[97]. The latter model allows evaluation of drug candidates aimed at treating this life-threatening condition because it provides a window onto pathophysiology that occurs deep inside living organs.

Application of low levels of fluid shear stress similar to that observed within the collecting ducts and proximal tubules of the living kidney enhanced differentiation (e.g., epithelial cell polarization, formation of primary cilia), increased molecular and drug transport functions, and produced more *in vivo*-like toxicity responses when primary rat[46], dog[72] and human[73] cells derived from these tissues were cultured in chips. More recently, a kidney-on-a-chip lined by immortalized human proximal

tubule cells was used to recreate the epithelial-to-mesenchymal transition that causes renal interstitial fibrosis during the development of proteinuric nephropathy[98]. This study revealed that exposure of renal epithelial cells to flowing medium containing human serum proteins or complement C3a induced apoptosis or a mesenchymal phenotype, providing new insight into this pathological process. The shape of a microchannel also can be altered to model disturbed flow. For example, a narrowed or stenotic region in an endothelial cell-lined microchannel provided insight into how the abnormal hemodynamics around an atherosclerotic plaque promotes thrombus formation[99].

Combining fluid flow and mechanical forcing regimens similar to those found *in vivo* can improve tissue- and organ-specific functions. Application of dynamic hydraulic compression to human bone marrow– and adipocyte-derived stem-cells-on-chips increased bone differentiation as measured by osteogenic gene expression and production of bone-inducing ECM components[50]. Human intestinal tumor–derived Caco-2 cells grow as poorly differentiated, flattened epithelial monolayers with low permeability barriers in static culture systems, but they are frequently used by pharmaceutical scientists to estimate intestinal barrier function because there are few alternatives. When Caco-2 cells are cultured on a flexible, porous ECM-coated membrane within a microfluidic device exposed both to trickling flow, analogous to that in the gut lumen, and to cyclic mechanical distortion, which mimics peristalsis-like motions of the living intestine, they reorganize into 3D undulating tissue structures lined by columnar epithelial cells that resemble the architecture of the villus of the small intestine[48,49] (work by D.E.I. and colleagues). Their relevant specialized features include reestablishment of functional basal proliferative cell crypts, differentiation of all four cell lineages of the small intestine types (absorptive, mucus-secretory, enteroendocrine and Paneth), secretion of high levels of mucin and formation of a higher-resistance epithelial barrier. In addition, it was possible to culture the human intestinal cells with living commensal bacteria in the lumen of the gut-on-a-chip without compromising cell viability, which opens a new avenue for microbiome research.

Microengineering has also been applied to model and measure the mechanical activities of contractile cells and tissues on chips. For instance, lines of ECM molecules patterned on thin layers of flexible PDMS by microcontact printing support adhesion of muscle cells and promote formation of oriented and highly contractile muscular tissue thin films *in vitro*[100,101] (work by S.N.B. and colleagues). When these thin films are partially released from the substrate, passive and active tension generated by cardiac, striated and smooth muscle cells can be quantified by measuring deformation of the flexible films[78,81]. This approach has been adapted to create a model of cardiac failure by mimicking mechanical overload[102], and to model the mitochondrial cardiomyopathy of Barth syndrome using patient-derived iPS cells and gene deletion in normal cardiomyocytes[103]. The recent demonstration that muscular thin films can be integrated into a microfluidic device[35] (**Fig. 2c**) raises the possibility of exploring how fluid flow, tissue-tissue interactions, and other mechanical and electrical cues contribute to cardiac disease development. A simpler microfluidic model of heart ischemia/perfusion injury has been created by subjecting primary porcine cardiomyocytes cultured in a microchannel under flow to periods of hypoxia followed by normoxia[40].

Integrating ECM and synthetic-polymer gels used in 3D culture systems into microfluidic channels provides a way to incorporate greater complexity of the tissue microenvironment on chips. For example, hepatocytes and fibroblasts were co-cultured on a microtextured surface to produce uniform cellular aggregates that were encapsulated in a synthetic 3D hydrogel using a microfluidic droplet generator to form hepatic microtissues[104] (work by S.N.B. and colleagues). These microtissues were introduced into a chip (**Fig. 4a**) under flow and exposed to drugs. Such microtissues have the advantage that cell states can be nondestructively sampled within the intact device by harvesting individual microtissues over time[104,105].

Multiple on-chip models of angiogenesis and microvascular function have been created as well. One includes a 3D stroma permeated by functional capillary networks that can sprout freely[106–108], whereas another model used biodegradable chips composed entirely of ECM that contained internal networks of microchannels filled with sacrificial material, which was dissolved before plating cells either inside the channels (endothelial cells) or in the surrounding perfused ECM (e.g., tumor cells, fibroblasts)[37,38] (**Fig. 3a**). This latter model is interesting in that newly formed microvessels become perfused once they functionally integrate into the existing vascular network. Such angiogenesis models enable high-resolution analysis of how spatial diffusive gradients influence angiogenic sprouting, and they have shed light on the mechanism of action of angiogenesis inhibitors[37,38,106–108]. A chip with a central, endothelium-lined channel separated by an ECM gel from a side channel containing chemoattractants was used to study transendothelial migration of neutrophils in response to chemical gradients[39] (**Fig. 3b**). A more recent development is the fabrication of multiplexed arrays of nearly identical ECM gels containing well-formed human microvascular networks on a single chip, which may be useful to study the effects of multiple perturbations on angiogenic responses[109].

Modeling organ-level physiology and disease. Although culture of a single cell type can mimic some facets of the tissue microenvironment on chips, it is not usually sufficient to generate organ-like functionality. An organ is a hierarchical structure composed of two or more different

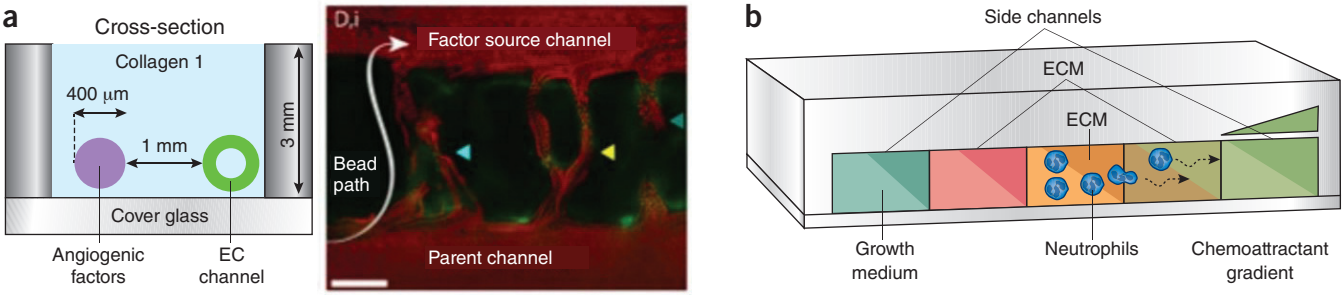

Figure 3 Microfluidic chip models of angiogenesis and immune cell invasion that incorporate ECM gels. (**a**) Sacrificial materials are deposited in linear patterns in an ECM gel and later removed to create two channels (left). One is populated with vascular endothelial (EC) cells[38], and the other is used to deliver angiogenic factors. Angiogenic stimuli induce the endothelial cells to undergo sprouting angiogenesis and then functionally link to the source channel, forming new microvessels that support fluid flow (right). Red indicates microparticles flowing in the medium. Fluorescence image reprinted from ref. 38 with permission. (**b**) A gradient-generating microfluidic culture device for analyzing immune cell migration through ECM gels when stimulated with a chemotactic gradient[39].

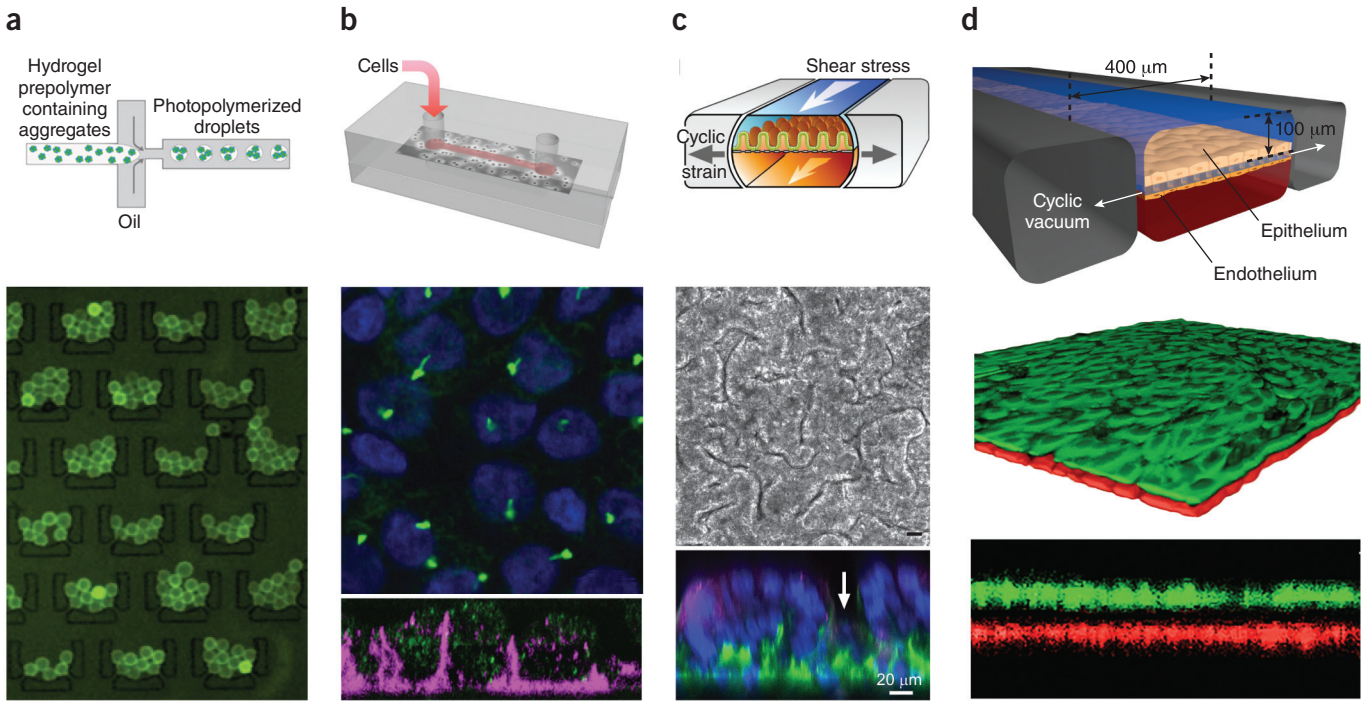

Figure 4 Examples of increasingly complex organ-on-chip designs. (**a**) A liver-on-a-chip in which hepatic microtissues composed of microscale hydrogels containing hepatocytes and fibroblasts are microengineered in one microfluidic system (top) and then used to populate another chip for culture and real-time multiplexed analysis (bottom)[104]. (**b**) A kidney-on-a-chip in which human kidney proximal tubular epithelial cells are cultured on the top of a porous membrane separating two channels, enabling analysis of transcellular transport, uptake and secretion (top)[73]. The upper fluorescence image of the epithelium shows enhanced formation of primary cilia (green) on the apical cell surfaces; the lower fluorescence cross-sectional view shows repolarization of Na^+K^+ ATPase (magenta) to the basal side. Fluorescence images reprinted from ref. 73 with permission of The Royal Society of Chemistry. (**c**) A gut-on-a-chip in which human Caco-2 intestinal epithelial cells are cultured on top of an ECM-coated, porous PDMS membrane separating two channels. Application of cyclic suction to side chambers mimics peristalsis (top)[48,49]. The phase-contrast micrograph shows a large region of the culture with undulating structures reminiscent of intestinal villi; the bottom fluorescence view shows a cross-section of this crenulated epithelial monolayer confirming the presence of crypts (arrow) separating adjacent villi. Fluorescence image reprinted from ref. 48 with permission of The Royal Society of Chemistry. (**d**) A 'breathing' lung-on-a-chip that recapitulates the alveolar-capillary interface. Human alveolar epithelial cells are cultured on top of a flexible, porous, ECM-coated membrane and human capillary endothelial cells on the bottom. Air is passed through the upper channel to create an air-liquid interface with the alveolar epithelium, and culture medium is flowed through the vascular channel, with or without human immune cells (top)[44,45]. Breathing motions are mimicked by applying cyclic suction to full-height side chambers that rhythmically distort and relax the flexible PDMS side walls and attached porous membrane. The fluorescence confocal 3D reconstruction at the bottom and the cross-sectional view shown at higher magnification in the inset show the tissue-tissue interface formed between the alveolar epithelium (green) and the endothelium (red). Fluorescence images reprinted from ref. 44 with permission of AAAS.

tissues, which are themselves made up of groups of different types of cells. An essential step toward replicating organ-level functions is combining two or more different tissue types, and this area has seen major advances in recent years.

A human blood-brain-barrier-on-a-chip was developed by lining a porous, fibronectin-coated polycarbonate membrane with human brain microvascular endothelium on one side and human astrocytes on the other[47]. The device included embedded microelectrodes to measure trans-epithelial electrical resistance (TEER) across the barrier. Notably, TEER levels were tenfold higher in this chip than those of the same cells grown on opposite sides of a polycarbonate membrane in a conventional static Transwell culture system. Co-cultures on the chip also were more impermeable to large molecules than monocultures of endothelial cells. Although the barrier function achieved was only ~25% of that observed in living brain microvessels, the device is superior to conventional culture models for measuring the permeability barrier of the central nervous system, and it may have great value for studying how drugs cross the blood-brain barrier.

A more complex microfluidic model of the neurovascular unit was created by placing rat brain microvascular endothelial cells on one side of a porous membrane and a mixture of three different brain cell types—astrocytes, neurons and microglia—on the other[94]. The endothelium retained good barrier function, and the neural cells fired inhibitory as well as excitatory potentials during 10 d of culture. Neuroinflammation was studied on chips by stimulating the vascular endothelium with TNF-α, which activated adjacent microglia and astrocytes, similar to what occurs *in vivo* in situations such as neuroinfectious disease. Another study modeled synapse formation in neurons and glial cells in close proximity (<100 μm)[89]. The presence of glial cells had a profound effect on the number and stability of synaptic contacts[89]. Synapse formation at the neuromuscular junction was also examined in chips containing mouse embryonic stem cell–derived motor neurons and C2C12 myotubes[90]. In addition, myelination has been studied on chips by co-culturing Schwann cells derived from human embryonic stem cells with human axons[91].

Co-cultures of different neural cells have been used to model neurological diseases on chips. Neurons and astrocytes genetically modified to overexpress either the wild-type or a mutated form of human super oxide dismutase enzyme 1 (SOD1) associated with amyotrophic lateral sclerosis, were cultured in close proximity but without permitting cell-cell contact[110]. Astrocytes with wild-type SOD lowered oxidative stress in adjacent cortical neurons, and neurons in metabolic contact with SOD-mutant astrocytes exhibited greater injury in response to severe glutamate treatment.

A human 'breathing' lung-on-a-chip recapitulated the alveolar-capillary interface using two channels separated by a porous ECM-coated

membrane[44] (work by D.E.I. and colleagues). In the lower microvascular channel, the membrane was lined with lung microvascular endothelial cells; in the upper, air-filled channel, it was lined by human lung alveolar epithelial cells. The two channels were bordered by full-height hollow chambers through which cyclic suction was applied to rhythmically distort the flexible PDMS side walls and the linked central porous membrane with attached cells in order to mimic the cyclic mechanical strain (10%; 0.25 Hz) that cells experience in the alveolus from breathing motions (**Fig. 4d**). The combination of fluid flow in the vascular channel, generation of an air-liquid interface in the alveolar channel and application of cyclic mechanical strain strongly promoted differentiation of the epithelial and endothelial cells lining the channels, as indicated by enhanced surfactant production and vascular barrier function (measured by both TEER and assays of macromolecular transport). When primary human neutrophils were flowed through the vascular channel under baseline flow conditions, the endothelium was quiescent. But addition of either the immune activator TNF-α or bacterial cells to the alveolar channel to mimic inflammation rapidly activated the endothelium, as measured by increased expression of surface ICAM-1 and recruitment of human neutrophils perfused through the vascular channel. The recruited immune cells underwent diapedesis and migrated through both cell layers to the upper chamber, where they engulfed the living bacteria. Because the cells were precisely positioned in the optically clear chip, these analyses could be carried out in real-time using high-resolution optical, fluorescence and confocal microscopy as well as microfluorimetry.

Because the lung-on-a chip mimicked complex organ-level functions, it enabled new mechanistic insights into the role of mechanical breathing motions in lung disease. When silica nanoparticle simulants of environmental air-borne particulates were introduced into the air channel, production of reactive oxygen species, cellular uptake of nanoparticles, and transport of nanoparticles across both cell layers and into the vascular channel were many-fold higher if the cells were experiencing cyclic breathing motions. This dependence of nanoparticle transport on breathing movements was confirmed in a mouse *ex vivo* ventilation-perfusion model[44]. The lung-on-a-chip was also used to model pulmonary edema ('fluid on the lungs') by perfusing through the vascular channel the cancer drug interleukin-2 (IL-2), which induces pulmonary vascular permeability and lung edema as its major dose-limiting side effect (D.E.I. and colleagues, ref. 45). When IL-2 was added at the same dose used in patients, pathological shifts of fluid into the air channel (**Fig. 4d**), formation of blood clots and associated compromise of oxygen transport occurred over the same 2- to 4-d time course as observed when the drug is administered in patients. The lung-on-a-chip also revealed that the mechanical forces of breathing motions contribute to the development of increased vascular leakage and pulmonary edema induced by IL-2, and that circulating immune cells are not required for the development of this life-threatening condition[45]. These results highlight the value of being able to vary cell types, chemical stimuli and mechanical perturbations independently in organs-on-chips.

Cancer-on-a-chip. Many groups have developed cancer-on-a-chip models. In a study of breast cancer progression, co-culture of human mammary epithelial cells with human mammary fibroblasts promoted a transition from ductal carcinoma *in situ* to invasive ductal carcinoma only when direct contact between the cells was permitted[111]. Effects of specific types of ECM on tumor-cell morphology and growth were detected when human breast carcinoma cells were co-cultured with stromal cells and various combinations of ECM components[112]. Taking advantage of the ability to install many different microenvironments on the same chip, another group discovered a correlation between the growth of breast cancer cells and ERα protein downregulation[113]. A similar but higher-throughput approach was used to fabricate tunable cell microniches and to carry out flow-based analysis of large cell populations to measure differential responses of lung adenocarcinoma cells to various ECM molecules and soluble factors. This work led to the finding that tumor-cell growth is sensitive to TGF-β and TGFβR2 inhibitor drugs in 3D microenvironments but not in monolayer culture[42]. Use of a microfluidic vascular endothelium model that permitted site-specific stimulation with the chemokine CXCL12 from the basal side of the tissue layer under variable flow conditions revealed that CXCL12 acts through CXCR4 receptors on the endothelium, rather than on tumor cells, to promote adhesion of circulating breast cancer cells[114]. Tumor-cell invasion also has been studied by measuring invadopodia formation and ECM degradation by human non-small cell lung cancer cells growing in an ECM gel–filled microfluidic channel[115]. Both intravasation[116] and extravasation[107,117] of tumor cells have been analyzed in chip models of angiogenesis as well.

Taken together, the research described above provides strong evidence that organs-on-chips are capable of reproducing human organ physiology and organ-level features of disease at both the population and individual levels. Because they allow different features of a cell culture, such as relative cell and tissue position, fluid flow and mechanical cues, to be controlled independently, they also provide unprecedented flexibility in dissecting the cellular, molecular, chemical and physical contributors to tissue and organ function, as well as disease development.

How might organs-on-chips be used for drug development?

ADMET testing and PK/PD modeling. The first major step in adapting microphysiological systems for drug development and toxicology came in 2004, when the Shuler group created a microfluidic cell-culture analog of a mathematical pharmacokinetics (PK) model that represents the organs of the body as interconnected compartments[43,63]. The purpose of this microfluidic analog was to study the adsorption, distribution, metabolism, elimination and toxicity (ADMET) of chemicals entirely *in vitro* rather than in animals, as is usually done in the pharmaceutical industry[43,63]. The same group has since created many types of microscale devices[43,63,74,118,119] that contain multiple culture chambers, each holding a single cell type representing a different organ (e.g., lung, liver, fat, marrow, tumor), and which are linked by microfluidic channels (**Fig. 5a**) in an order that mimics physiological coupling *in vivo* (**Fig. 5b**). When the toxicant naphthalene was introduced into one of these devices, it converted in the liver chamber into its reactive metabolites, which then circulated to the lung tissue chamber where they depleted cellular glutathione levels[43,63]. Adipocytes in the fat chamber moderated the glutathione depletion induced by naphthalene and preferentially accumulated hydrophobic compounds[43,63]. Thus, this simple chip mimicked basic organ-organ coupling and permitted analysis of drug ADMET characteristics that are usually determined *in vivo*. These early studies were limited by the use of cultured cell lines that were from different species and were not optimally differentiated. Another potential problem is that the interstitial fluids were flowed directly from one cell compartment to the other, which does not occur in distant living organs coupled by the vascular system. Nevertheless, these reports first raised the intriguing possibility of developing a 'human-body-on-a-chip'—a microfluidic device containing several fluidically linked chambers representing different organs—that could be used to test drugs, chemicals or toxins.

Over the past ten years, many groups have explored the use of organs-on-chips to study drug ADMET properties, to support PK

and pharmacodynamics (PD) modeling, and to measure drug efficacy. For example, livers-on-chips are useful for analyzing drug metabolism by liver tissue. In one study, HepG2/C3A cells exposed to the anticancer prodrug flutamide or to its active metabolite (hydroxyflutamide) were analyzed on a chip using nuclear magnetic resonance (NMR) spectroscopy to identify response markers[120]. The prodrug and the active compound produced distinct metabolic signatures and hepatotoxicities, which correlated with effects on glutathione metabolism. NMR-based metabolomic footprinting was also used with chips as a high-throughput approach to determine the toxicities of several small molecules, including the environmental pollutant ammonia, the free radical-scavenging solvent dimethylsulfoxide and the hepatotoxic analgesic drug *N*-acetyl-para-aminophenol (acetaminophen or paracetamol)[121]. This effort identified signatures for ammonia and acetaminophen and dose-dependent metabolic responses to ammonia in chambers lined by liver (HepG2/C3A) cells, kidney cells and co-cultures of both cell types. However, another study on a liver-kidney chip found that HepRG cells derived from a human liver progenitor cell line metabolized ifosfamide into its toxic metabolite chloroacetaldehyde, whereas HepaG2/C3A cells did not[122], which emphasizes the importance of cell selection[123,124].

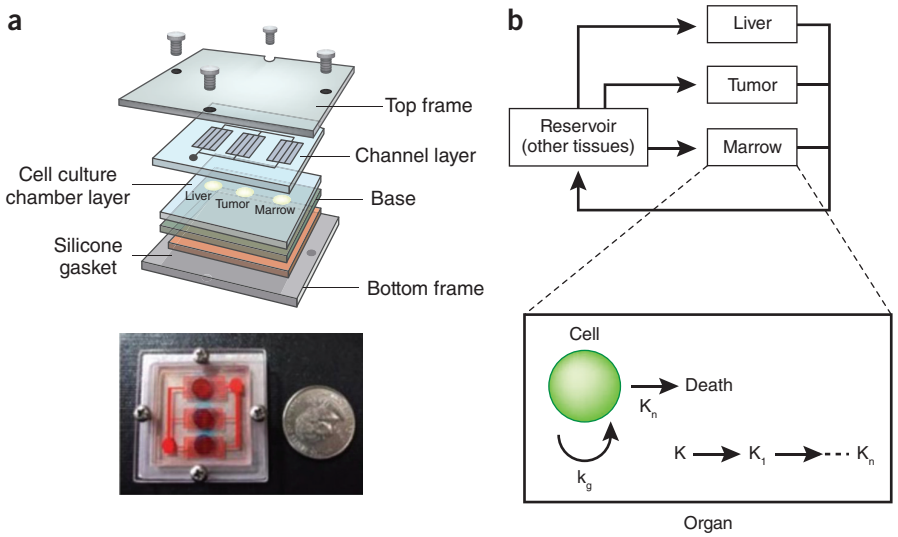

Figure 5 A multi-organ microfluidic framework used for PK/PD modeling. (**a**) Schematic diagram (top) and photograph (bottom) of a three-chamber chip used for PK modeling by flowing medium through liver, tumor and marrow cells cultured as monolayers in separate chambers and linked fluidically. (**b**) A flow diagram of the connections between the liver, tumor and marrow compartments in the chip shown in **a** (top), and a pharmacodynamics model for cell death in each compartment (bottom). Reprinted from ref. 156 with permission of The Royal Society of Chemistry.

Some ADMET properties of acetaminophen were also demonstrated by coupling a microchamber lined by a monolayer of liver (HepG2/C3A) cells to a macroscale (5.6 mm high including top and bottom portions) chamber containing a mixture of intestinal cells[74]. Administration of acetaminophen resulted in glutathione depletion in the intestinal cells and further metabolic processing by the liver cells, causing dose-dependent hepatotoxicity. The experiment was designed so that the drug residence time in the liver chamber was similar to that measured in human liver (~1–2 min). These results were consistent with *in vivo* measurements.

Although tumor-derived hepatic cell lines were used to model liver metabolism and function in the multi-organ chips described above, more recent work has demonstrated that primary human hepatocytes are superior for predicting drug metabolism, clearance, drug-drug interactions and toxicity. Normal human hepatocytes cultured on chips maintained the functionality of multiple clinically relevant liver cytochromes P450 (CYP1A2, CYP3A4, CYP2C8, CYP2C19 and CYP2D6)[125] and enabled measurement of hepatic clearance of six marketed drug compounds[64]. In the latter study, application of flow increased the duration of metabolic competency compared with static culture, consistent with other microfluidic studies using primary hepatocytes[126], but hepatocytes under flow could not distinguish high- and medium-clearance compounds, and neither model detected low-clearance compounds. Clearance of drug compounds with high, medium and low clearance values can be improved by culturing primary hepatocytes with nonparenchymal cells under flow[70].

Unfortunately, despite these advances, livers-on-chips still cannot capture certain key hepatic functions, such as directional biliary ductal clearance or sustained production of metabolic enzymes and blood proteins for the typical hepatocyte life span of approximately one year *in vivo*. In fact, in some cases, the presence of flow may actually be detrimental to predictivity. For example, the US Food and Drug Administration (FDA) guidance on Metabolites in Safety Testing notes the importance of detection of certain human metabolites that so far has relied on metabolite accumulation in small static environments owing to the sensitivity limitations of analytical methods[127].

In certain chip designs, recirculating flow can effectively make them 'well stirred' models. This enables scaling of obtained clearance data to estimate human hepatic clearance without mathematical modeling, which is often required in conventional hepatocyte cultures or suspensions. For example, when hepatic clearance rates measured under flow on a chip or under static conditions were compared with *in vivo* data from the literature, both formats generated predictions on the same scale as the reported values[64]. But the chip predictions did not require mathematical modeling, at least for this limited set of compounds. In this study, and in another one using human hepatocytes that found consistent results[128], nonspecific adsorption of drugs into walls and tubing complicated the calculation of clearance rates. This remains a major problem for chips made of PDMS.

Microfluidic coupling has also been shown to be relevant for analysis of cancer drug metabolism and toxicity. For example, a study involving linked chambers containing liver (HepG2/C3A), bone marrow (MEG-01), uterine cancer (MES-SA) and a multidrug-resistant (MDR) variant of uterine cancer (MES-SA/DX-5) cells found that combining the chemotherapeutic drug doxorubicin (Adriamycin, Doxil) with MDR modulators (cyclosporine and nicardipine) produced greater antiproliferative effects on the MDR cancer cell variant than any of these compounds alone; and, this result could not be obtained using a conventional static culture[118]. In another study, HCT-116 colon cancer cells (HCT-116) and hepatoma cells (HepG2/C3A) were encapsulated in an ECM gel (Matrigel) and cultured in linked tumor and liver chambers on-chips[119]. This microfluidic system was able to reproduce the metabolism of an oral prodrug of the anticancer drug, 5-fluorouracil (Carac, Efudex, Fluoroplex), to its active product in the liver chamber, and result in killing of the tumor cells when the metabolite flowed to the other chamber, whereas, again, cultures of similar cells in a 96-well microtiter plate did not.

Chips are also well suited to the study of drug transport and toxicity. Data from a blood-brain-barrier-on-a-chip analyzing the

permeability of a set of hydrophobic and hydrophilic drugs correlated well with corresponding *in vivo* values[92]. As discussed above, the human lung-on-a-chip permitted analysis of silica nanoparticle transport across the alveolar-capillary interface and detection of the increase of vascular permeability induced as a toxic side effect by the human anticancer drug IL-2, in addition to revealing the key role of physiological breathing motions in these responses[44,45]. Neither of these results had been reported previously, and it would be difficult to generate them using standard cell cultures, macroscale bioreactors or animal models.

A major problem with preclinical animal studies is that drug toxicities are difficult or impossible to identify if there are meaningful differences in drug uptake between the animal model and humans. In such cases, human cells or tissues are needed. For instance, the anticancer drug cisplatin (Platinol) has different toxicities in animals and humans owing to species differences in membrane transporters, such as OCT2, which mediate cellular accumulation of this drug and production of reactive oxygen species. A human kidney-proximal-tubule-on-a-chip measured OCT2-specific cisplatin toxicities that were undetectable in a static culture (work of D.E.I., ref. 73). The chip also measured the activity of P-glycoprotein ATP-binding cassette membrane (Pgp) transporters that mediate the efflux of certain cancer chemotherapeutic drugs in humans and thereby produce multidrug resistance. This system may provide a valuable *in vitro* screen for drug development given that new draft guidelines of the FDA require determination of whether a drug candidate is a substrate or inhibitor of Pgp transporters as drug interactions with Pgp can increase the toxicity of co-administered agents.

Efficacy testing and drug discovery. Organs-on-chips are beginning to be applied to assess drug efficacy as well. For example, a heart-on-a-chip, containing almost 20 rat cardiomyocyte thin films, stimulated electrically to contract, was used to test the inotropic effects of the beta-adrenergic agonist isoproterenol[35]. The results were similar to those previously determined in rats. The chip also allowed multiplexed, high-resolution imaging of cell structure and function, as well as quantification of the effects of the drug on diastolic, systolic and twitch stress. In a related study, responses of muscular thin films constructed from cardiomyocytes or vascular smooth muscle cells to endothelin-1 and rho-associated kinase (ROCK) inhibitor were analyzed simultaneously on the same chip[81]. A high dose of endothelin-1 increased vascular smooth muscle contractility while suppressing cardiomyocyte contraction, and the ROCK inhibitor decreased tension in both types of muscular thin films. These findings show that the effects of drugs on different tissue types can be analyzed simultaneously on the same chip, which could improve assay throughput relative to animal studies or conventional cell cultures.

The effects of the clinical surfactant drug beractant (Survanta) were studied in a microfluidic pulmonary airway model that enables analysis of damage to the epithelium during reopening of airways occluded by liquid plugs (analogous to mucus plugs)[77]. These studies revealed that substantial cellular injury occurs owing to high mechanical stresses caused by plug propagation devoid of surfactant, whereas addition of a physiologic concentration of Survanta protected the epithelium and significantly reduced cell death. Computational simulations also revealed a significant decrease in mechanical forces in the presence of surfactant, confirming the experimental observations.

Recent studies also raise the possibility of using organs-on-chips to discover new therapeutics or new uses for existing ones. When a current drug candidate that inhibits TRPV4 ion channels and suppresses mechanosensing in endothelial cells[129] was tested in the lung-on-a-chip, it completely inhibited IL-2–induced human pulmonary edema[45], and confirmatory results were obtained in a cardiogenic pulmonary edema model in rodents and dogs[130]. The ability to culture a living microbiome in the human gut-on-a-chip revealed that co-culture of probiotic bacteria (*lactobacillus GG*) with human intestinal epithelial cells increases intestinal barrier integrity[48], suggesting that this chip might be used to test the efficacy of other probiotic therapies. A recently developed bone-marrow-on-a-chip that permits culture of fully formed living marrow with a functional hematopoietic niche within a chip was used to model both organ-level marrow toxicity responses to radiation and the protective effects of radiation countermeasure drugs, whereas similar effects could not be measured with conventional bone marrow culture methods[84] (work by D.E.I. and colleagues). Screening for drugs to protect against exposure to lethal radiation is an excellent example of how organs-on-chips can enable studies examining human responses that would otherwise be impossible to carry out in human patients due to ethical implications.

Cancer-on-a-chip devices also show promise for drug screening. Functional interrogation of hundreds of single chronic myeloid leukemia cells or normal hematopoietic stem cells allowed detection of tumor-specific cellular responses to the tyrosine kinase inhibitor dasatinib (Sprycel), approved for the treatment of this cancer[131]. In another study, the responses of a lung cancer cell line, a mixture of lung cancer and stromal cell lines, and cells from fresh lung cancer tissues were compared when cultured in 3D gels and exposed to different concentrations of chemotherapeutic agents generated on-chip by a concentration-gradient generator[132]. The investigators assayed the sensitivities of different anticancer drugs in parallel and screened appropriate-dose, single-drug and combined-drug chemotherapy schemes for eight patients. Another example of work with potential relevance to personalized medicine involved use of a marrow-like environment containing human osteoblasts to culture mononuclear cells from clinical bone marrow aspirates as a way to expand patient-specific multiple myeloma cells that could be used to screen for individualized therapies[83].

What are the advantages and disadvantages of organs-on-chips?

Conventional 3D culture systems—including hydrogel-based methods, tissue-engineered constructs, static co-cultures, and bioreactors—have proved very useful for studying certain tissue- and organ-level behaviors and for developing disease models. Examples include mammary[4], intestinal[5] and brain[6] organoids; co-cultures of hepatocytes with fibroblasts or endothelial cells in Petri dishes or bioreactors[68,69,96,133–135]; micro–cardiac muscles for measuring response to contractile modifiers[78,136] and modeling heart failure and cardiomyopathy[102,103]; atrophic skeletal muscle[137] and mechanosensory circuits containing innervated muscle fibers[138]; and models of liver infection by hepatitis C virus[139] and *Plasmodium falciparum* and *Plasmodium vivax*[140]. Macroscale culture chambers or bioreactors have been connected fluidically, either directly or through endothelium-lined channels to link multiple engineered mini-organs (e.g., liver, skin, hair, brain, bone marrow, lymphatic) for drug testing[68,69,141–145], much like in microfluidic systems. These systems generate more tissue mass compared with microfluidic chips, which is advantageous when using analytical approaches (e.g., mass spectroscopy) that require larger experimental samples. It is also easier to retrieve larger numbers of cells for analysis from larger systems. Some organ functions—such as cognition in the brain and mechanical function in bone, ligaments and tendons—arise out of macroscale architecture and cannot be readily modeled on chips, although parts or sections of organs (e.g., artery[146], pancreatic islets[147], liver, intestine and brain[148,149]) have been cultured on chips to study certain organ-level functions and responses to chemical or electrical signals. It is also difficult to reproduce on a microscale the spatial heterogeneity found in larger 3D organoids or tissue sections (e.g., in the lung, the spatial transition in epithelial structure from tracheal to bronchial to respiratory to alveolar).

Microfluidic organ-on-chip research must also contend with specific technical challenges. Fabrication requires specialized micro-engineering capabilities. Bubbles in microfluidic channels may injure cells and hamper fabrication and control of chips, and it can be difficult to completely remove them. Although continuous perfusion generally supports high levels of long-term cell survival, the use of simplified ECM gels or thin ECM coatings can be a problem owing to matrix degradation or contraction over time. Additional challenges include achieving robust, consistent cell seeding in microfluidic channels, preventing microbial contamination, and controlling the cell-cell and cell-ECM interactions necessary to generate precise tissue structure-function relationships.

But even with these limitations, microfluidic culture devices have much to offer. One drawback of macroscale 3D culture systems is that as their functionality and complexity increase, it becomes harder to carry out high-resolution imaging and to determine where in the tissue to look, much as it is difficult to visualize processes in living organs. In organs-on-chips, cell types of one tissue can be positioned precisely and consistently relative to those of another. This is why it has been straightforward to integrate these systems with fluorescence confocal microscopy, microfluorimetry, TEER measurements, multiple electrode arrays and many other on-line analytical assays. In the future, it should be possible to incorporate molecular reporters and nanoscale sensors and to extend time-lapse imaging to groups of linked organs-on-chips using multiplexed microscopes or robotic systems. Scaling up to larger numbers of samples can also be easier than in macroscale systems, which could increase the statistical significance of results.

Another advantage of organs-on-chips is the ability to control fluid flow, which enhances the differentiation, function and long-term survival of many cell types. For example, human lung cells have been cultured on chip in a functional state for at least one month in culture[44]. This capability could enable future studies to be carried out on clinically relevant time scales of chronic pathophysiological responses. For example, many forms of hepatotoxicity emerge over weeks to months rather than acutely over 24 h, as hepatotoxicity is often modeled[150]. Inclusion of flow allows testing of microenvironmental chemical signals, such as chemical, oxygen and cytokine gradients, as well as hormonal (soluble signals between organs) and angiocrine (soluble signals from endothelium) cues. Flow also enables organs-on-chips to model ADMET properties, which makes prediction of PK/PD properties of drugs more accessible than with static systems. In addition, the well-defined architecture of organs-on-chips facilitates computational modeling of the fluid dynamical interactions among different gases, metabolites and cells, which invariably affect cell viability and function. Finally, inclusion of flow permits the study of interactions with circulating cells, such as various types of blood, immune and tumor cells, or with bacterial cells, as in a microbiome–intestinal cell system[48].

The true power of microsystems engineering lies in the ability to design synthetic culture systems in which many different control parameters (e.g., types and positions of cells; precise 3D orientation of tissue-tissue interfaces; transcellular chemical, molecular and oxygen gradients; flow levels and patterns; mechanical forcing regimens) can be varied independently while simultaneously carrying out high-resolution, real-time imaging of molecular-scale events within a 3D tissue or organ context. This unprecedented level of control makes it possible to replicate distinct functional units of organs (e.g., heart vs. lung; lung alveolus vs. bronchiole vs. bronchus vs. trachea). Eventually, such functional organ units, each in its own chip, could be linked by vascular or interstitial channels (e.g., vascular channel of lung to heart; interstitial channels of trachea-bronchi-bronchiole-alveolus) to create synthetic models of whole organs. Multiple organ models could then be connected in physiologically relevant orders by an endothelium-lined, microfluidic vasculature to simulate a 'human body-on-a-chip', which can be instrumented to enable automated time-lapse microscopic imaging in multiple organs simultaneously, and application of existing analytical tools used for characterization of live cell cultures. One can also envision channels lined by lymphatic endothelial cells to create lymphatic drainage. This approach differs from most existing multi-organ microscale and macroscale models, which link interstitial (parenchymal) tissue chambers by inert conduits.

Outlook

The potential for transformative change. As an alternative to conventional cell culture and animal models, human organs-on-chips could transform many areas of basic research and drug development. They could be applied to research on molecular mechanisms of organ development and disease, on organ-organ coupling and on the interactions of the body with stimuli such as drugs, environmental agents, consumer products and medical devices. Fundamental questions that might be addressed include how microenvironmental cues regulate cell differentiation, tissue development and disease progression; how tissues heal and regenerate (e.g., mechanisms of control of angiogenic sprouting and epithelial sheet migration); and how different types of immune cells and cytokines contribute to toxicity, inflammation, infection and multi-organ failure. When combined with patient-specific primary or iPS cells, or with gene editing technologies (e.g., CRISPR) to introduce disease-causing mutations[103], this technology could be used to develop personalized models of health and disease.

Organs-on-chips are currently not well suited to certain areas of biomedical research, such as chronic diseases, adaptive immune responses, or complex system-level behaviors of the endocrine, skeletal or nervous systems. As described above, they are effective for investigating physiological and disease processes that occur in a relatively short time frame (less than ~1 month) and depend on relative cell positions within an organ- or tissue-specific microarchitecture. Taking the liver as an example, one can study many facets of hepatic physiology with livers-on-chips, such as production of liver-specific proteins (albumin, complement, clotting factors), polarized cell trafficking of lipids and other species, and energy metabolism across different patient populations. Stress responses, such as the acute phase response, as well as effects of ischemia, regional hypoxia and nutritional deprivation, can also be analyzed. Livers-on-chips show promise for investigating the interactions of these processes with drugs, including drug bioactivation, drug clearance, drug-drug interactions through induction/inhibition pathways, susceptibility to drug-induced liver injury, and production of reactive metabolites that can interact with other organs. However, it has still not been possible to integrate a functional biliary outflow tract into these devices or to sustain physiological function for months.

This approachable class of problems comprises dose-dependent, species-specific and relatively acute drug interactions. More difficult to study are idiosyncratic drug toxicity responses that emerge at an extremely low rate (e.g., 1/10,000) in the general population, which likely have an immune component and can emerge over weeks to months. Nevertheless, even these rare responses could be studied using livers-on-chips made from iPS cells derived from the rare patients with idiosyncratic responses. As drug toxicity also can have an immunological component, including immune cells in the perfusate of livers-on-chips could aid in the assessment of immune-mediated processes such as inflammation-induced acute-phase responses, suppression of drug metabolism and injury-induced immune cell infiltration. Establishing a functional biliary network is another important goal for liver-on-chip technologies as this

will afford the study of drug disposition across the basolateral cell surface to the apical domain—so-called phase III transport. Many diseases of the liver are chronic in nature, driven by stellate-cell activation and collagen remodeling that evolve over years. A subset of patients with fibrosis then progress to cirrhosis, a risk factor for hepatocellular carcinoma. Long-term processes such as these are less amenable to study in any *in vitro* format, although some of their features could be replicated on chips. For example, modeling the interactions of hepatocytes and peripheral adipose tissue in nonalcoholic fatty liver disease may offer valuable insights even if multiyear observation is not possible.

Directions for future research. There are many challenges that must be overcome before organs-on-chips will find widespread use in research laboratories. A basic problem that must be remedied is the material to use for fabrication. Most chips are made out of PDMS because it is easy to use and has high optical clarity, gas permeability and biocompatibility. But PDMS can absorb small organic compounds, including many drugs, and its high gas permeability can hinder some applications. Recently, other polymers, such as certain polyurethanes, were found that provide the benefits of PDMS but do not soak up small hydrophobic drugs[151]. However, more research is required to identify suitable materials that can be used to mass produce organs-on-chips at low cost. Another materials problem is that the ECM-coated PDMS membranes that serve as tissue-tissue interfaces may have transport, mechanical and structural properties different from those of natural basement membrane. Given issues such as these, it will be many years before the community can consider developing generic production specifications for organs-on-chips.

Another major challenge is technical robustness. Various factors must align to achieve optimal function of organs-on-chips over a month or longer, including the cells, the ECM coatings, fluidic control, bubble removal and gradient maintenance. For systems consisting of multiple, linked organ chips, there is a need for a 'universal blood substitute'—a single culture medium that supports all tissues, just as blood supplies all organs in the body. Existing culture media have been optimized for each cell type, so this remains a significant hurdle, especially if some tissues are grown in serum-containing medium and others in serum-free medium. In a study examining whether addition of growth factors, such as TGF-β, might enhance tissue functionality in a multiorgan chip containing chambers lined by liver, lung, kidney and adipose cells, the investigators discovered that this supplement enhanced the function of one cell type but inhibited the function of another[152]. They overcame this limitation by mixing controlled- release, gelatin microspheres containing TGF-β with the responsive cell type to create a local cell-specific microenvironment within the multiorgan system. Another possible solution is to flow either serum-containing medium, plasma or whole blood through microchannels lined by a vascular endothelial barrier that regulates delivery of molecular factors to adjacent interstitial channels, as occurs *in vivo*.

The presence of flow in microfluidic systems has already made it possible to develop PK models by combining data on clearance dynamics from single or coupled organs-on-chips with mathematical scaling approaches[43,63,125,153]. But there is a need for improved scaling approaches that more accurately match fluid flows, cultured tissue mass and volumes of distribution so as to ensure appropriate, relative, organ functional activity and to provide meaningful data for computational PK/PD models[154,155]. This goal would be more tractable with data from 'next-generation' chips that might include in-line sensors of critical control and functional parameters (e.g., flow, pressure, temperature, pH, oxygen, glucose, lactate, TEER, electrical conduction) and integrated microscopic and microfluorimetric imaging capabilities to monitor overall system performance in real-time[154,155]. But broad acceptance of organs-on-chips in basic research and drug development will likely require automated instrumentation that provides feedback control, continuous regulation, programmable monitoring, and experimental sample collection and processing. Currently, laboratories that wish to work with organs-on-chips require significant in-house engineering capabilities and expertise. Automated control systems would allow nonspecialist academic researchers to replace existing 2D or 3D culture systems with microfluidic systems that provide similar levels of molecular analysis but in a functional-organ context. For example, once samples are collected from vascular or interstitial microfluidic channels, they can be analyzed using virtually any current method, such as histocytochemistry, western blots, PCR, gene microarrays and mass spectroscopy.

The greatest value of organs-on-chips for the pharmaceutical and biotechnology industries may lie in the validation and prioritization of lead drug candidates (rather than in high-throughput screening) and in the study of molecular mechanisms of action and toxicities. Sophisticated human organs-on-chips may also help to identify new biomarkers of drug efficacy, toxicity or disease response, which could be of value for clinical trials. If clinically relevant PK/PD modeling approaches can be developed, it may be possible to use this technology to determine drug dosing and safety margins for clinical trials as well. As drug companies will surely continue to rely on preclinical animal studies for many years to come, it will also be important to develop organs-on-chips lined with cells from the animal species now used for drug development or with human cells to study species differences and to refine *in vitro–in vivo* correlations and predictions.

Although there have been a few recent successes demonstrating that organs-on-chips can mimic specific organ-level functions, the field is still in its infancy. If the technology can be improved to the point that it effectively recapitulates a broad range of organ responses to chemicals, drugs and toxins, many new avenues for drug discovery, toxicology and personalized medicine will open up. These include testing of dangerous agents (e.g., highly infectious viruses, biothreat agents, chemical warfare agents and lethal doses of γ-radiation) and 'virtual' pediatric clinical trials. In addition, organs-on-chips generated from iPS cells isolated from different genetic subpopulations, disease subgroups or individual patients might facilitate drug discovery targeted to specific subpopulations or clinical trial design.

Given the complexities of organ function and regulatory requirements, it is unlikely that organs-on-chips will replace animal testing anytime soon. However, as individual organs-on-chips are improved, it may be possible to progressively replace one animal-based assay at a time. Researchers in the field must recognize that there are major hurdles to overcome before this technology will have widespread acceptance and impact. But we cannot ignore its amazing potential, and we hope that others will share our excitement and jump into the fray.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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