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Organs-on-chips at the frontiers of drug discovery

Eric W. Esch,

Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Anthony Bahinski, and

Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, Massachusetts 02115, USA

Dongeun Huh

Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Abstract

Improving the effectiveness of preclinical predictions of human drug responses is critical to reducing costly failures in clinical trials. Recent advances in cell biology, microfabrication and microfluidics have enabled the development of microengineered models of the functional units of human organs — known as organs-on-chips — that could provide the basis for preclinical assays with greater predictive power. Here, we examine the new opportunities for the application of organ-on-chip technologies in a range of areas in preclinical drug discovery, such as target identification and validation, target-based screening, and phenotypic screening. We also discuss emerging drug discovery opportunities enabled by organs-on-chips, as well as important challenges in realizing the full potential of this technology.

The pharmaceutical industry is facing unprecedented challenges owing to rising costs and the declining efficiency of drug research and development[1,2]. As drug failures in clinical trials are primarily due to the poor predictive power of existing preclinical models, the drug discovery community has identified the critical need for new testing approaches to generate reliable predictions of drug efficacy and safety in humans[3–6].

Microengineered cell culture models could provide a solution to this unmet need. These models leverage cutting edge microfabrication and microfluidics technologies to control the cellular microenvironment with high spatiotemporal precision and to present a variety of extracellular cues to cultured cells in a physiologically relevant context[7–10]. The latest progress in this area has led to the development of novel microdevices, known collectively as organs-on-chips, that recapitulate the complex structure, microenvironment and physiological functionality of living human organs[11–13].

*Correspondence to D.H.*huhd@seas.upenn.edu.

Competing interests statement

The authors declare competing interests: see Web version for details.

In this article, we provide a perspective on the breadth of new opportunities afforded by the integration of this emerging organ-on-chip technology with drug discovery and development. We first introduce the technological background and advantages of organs-on-chips and then use examples drawn from recent studies to demonstrate how organ-on-chip technology has begun to tackle major technical challenges at the critical steps of conventional and emerging drug discovery processes in a cost-effective manner. Future directions and technical challenges for the field are also discussed.

Microphysiological organ models

Organs-on-chips are microengineered biomimetic systems that represent key functional units of living human organs. They often consist of transparent 3D polymeric microchannels lined by living human cells and replicate three important aspects of intact organs: the 3D microarchitecture defined by the spatial distribution of multiple tissue types; functional tissue–tissue interfaces; and complex organ-specific mechanical and biochemical microenvironments[11,13–17]. These systems could be used as specialized *in vitro* models that permit simulation, mechanistic investigation and pharmacological modulation of complex biological processes (TABLE 1).

In recent years, this biomimetic microsystems approach has been used to establish microengineered models that recapitulate the structural and functional complexity of human organs such as the liver, heart, lung, intestine, kidney, brain and bone[11,18–20]. A representative example is the lung-on-a-chip microdevice that reconstitutes the mechanically active alveolar–capillary barrier in the human lung[13] (FIG. 1). This model is created in a compartmentalized 3D microfluidic system in which human alveolar epithelial cells are cultured in close apposition with human pulmonary microvascular endothelial cells on a thin porous elastomeric membrane to form a barrier tissue that resembles the *in vivo* alveolar–capillary interface. This microfluidic cell culture system is integrated with a biologically inspired mechanical actuation system that uses computer-controlled negative pressure to cyclically stretch the alveolar–capillary barrier to mimic physiological breathing motions. Importantly, this device enables the reconstitution and visualization of complex, integrated, organ-level responses not normally observed in conventional cell culture models, such as recruitment and phagocytic activity of blood-borne immune cells in response to bacteria, inflammatory cytokines and environmental nanoparticulates[13] (FIG. 1b). Moreover, the ability of this model to recapitulate the dynamic mechanical activity of the lung has led to the discovery of previously unexplained adverse effects of physiological breathing-induced mechanical forces on inflammatory and injury responses. For example, in nanotoxicology studies using silica nanoparticles that simulated air pollutants, the cyclic breathing motions in the lung-on-a-chip system substantially increased the endothelial expression of pro-inflammatory adhesion molecules and the intracellular production of reactive oxygen species, indicating that physiological breathing promotes acute toxic responses to environmental particulates[13]. This biomimetic microdevice also revealed considerable increases in the translocation of nanoparticles from the alveolar airspace to the vascular compartment due to breathing-associated mechanical strain[13].

The same system was further engineered to model the development and progression of pulmonary oedema induced by toxicity of the anticancer drug interleukin-2 (IL-2)[15] (FIG. 1c). Specifically, clinically relevant concentrations of IL-2 administered into the microvascular channel led to continuous leakage of vascular fluid into the alveolar compartment and complete flooding of the airspace in time frames similar to those observed in humans. Based on the observation that these deleterious responses were greatly exacerbated by physiological mechanical stresses produced by breathing, this microengineered disease model also facilitated the identification of novel therapeutics that inhibited mechanotransduction pathways critical to compromised alveolar–capillary barrier function (this study is revisited in greater depth in the 'Toxicity screening' section below). Importantly, key findings in these studies were reproduced in whole mouse lung, demonstrating the capability of the lung-on-a-chip system to faithfully recapitulate and predict complex physiology and pathology *in vivo*[15].

Comparison with conventional models

For the evaluation of drug efficacy and safety, organs-on-chips provide compelling advantages over other *in vitro* cell culture models. Although 2D culture models can provide large amounts of relatively inexpensive data, these models poorly represent complex pathophysiology in patients and require computational modelling and systems biology approaches to predict *in vivo* drug responses.

Biomimetic 3D tissue structures with physiological barrier function more accurately simulate delivery and penetration of drug compounds *in vivo* than do 2D cell monolayers in conventional culture models[21] (TABLE 2). More importantly, these microengineered co-culture models recapitulate the complex interactions between different types of cells *in vivo* that are mediated by various soluble and insoluble factors such as cytokines, nutrients, growth factors, hormones, the extracellular matrix and intercellular junctions, all of which can be controlled within the organ-on-a-chip[20,22–24]. Unlike 2D culture systems, organs-on-chips can therefore reconstitute complex organ-level physiological functions, clinically relevant disease phenotypes and pharmacological responses that arise from structural and functional integration of multiple tissue types. This characteristic allows for more comprehensive and accurate predictions of complex drug responses *in vivo*. Fluid motions and cell culture substrates in organs-on-chips can also be controlled to reproduce various types of mechanical cues induced by physiological flow (for example, blood flow and interstitial flow) and tissue deformation (for example, breathing, peristalsis and heartbeat), including fluid shear stress, tension, compression and torque[11,22–25].

Organs-on-chips also have advantages over current animal models. Although animal studies can emulate physiological complexity at the whole-organism level, animal surrogates of human diseases are now facing increased scrutiny and scepticism regarding their scientific validity and translatability to humans[26,27]. Recent systematic studies on the predictive value of animal models have demonstrated a poor correlation between animal data and human outcomes owing to substantial interspecies differences in key disease pathways and disease-induced changes in gene expression profiles, highlighting the critical need for new approaches to model complex human-relevant conditions[28,29].

The optical transparency of organ-on-chip microdevices is another key advantage over animal models as it enables direct real-time visualization and quantitative high-resolution analysis of diverse biological processes in ways that have not been possible in animal models[30]. In addition, compartmentalized channel designs for co-culture in organs-on-chips (for example, upper and lower channels separated by a thin porous membrane) allow for independent fluidic access to different tissue types within a single device and parametric control of microenvironmental factors. Conditioned media can therefore be sampled from specific tissue types of interest to analyse their metabolites and other secretory products, which may aid in the identification and development of novel biomarkers for efficacy, toxicity or disease processes[25,31].

Organs-on-chips could also reduce consumption of costly reagents including cells, culture media and drug compounds. Considering that animal studies typically require scale-up of compound production to generate adequate quantities for dosing, organs-on-chips could be used earlier in the drug discovery pipeline than animal models, whether to more robustly characterize investigative compounds or to bridge conventional 2D culture studies.

Target identification and validation

Organs-on-chips have the potential to serve as a new enabling platform to identify and validate the efficacy, safety and druggability of potential targets early in the pipeline to increase the likelihood of success in clinical trials. A good example of such an application is provided in the work of Song *et al.*[32], whereby a microengineered model of vasculature was used to mechanistically examine chemokine-mediated interactions between circulating breast cancer cells and the microvascular endothelium (FIG. 2a). This microsystem consisted of an upper intravascular compartment and lower stromal chambers separated by a semi-porous membrane lined with human microvascular endothelial cells. The compartmentalized channel design permitted the recreation of physiological dynamic flow conditions and circulation of metastatic breast cancer cells in the vascular channel. This model also enabled site-specific basal stimulation and activation of the microfluidic endothelium by introducing chemokines into the lower chambers. Through quantitative analysis of cancer cell attachment to the endothelium and the levels of cell surface receptor expression, this system revealed that endothelial recruitment of breast cancer cells induced by CXC-chemokine ligand 12 (CXCL12), a chemokine involved in cancer metastasis, is mediated by the endothelial receptor CXCR4 and that this response is independent of the expression of CXCL12 receptors on circulating cancer cells. These findings demonstrated a previously unknown critical role of the vascular endothelium in the metastatic behaviour of circulating tumour cells. More importantly, the functionality of this model enabled the researchers to precisely control and manipulate a biological target and to analyse a functional outcome of target modulation, which led to the discovery that inhibition of CXCL12–CXCR4 binding on endothelial cells may be a valid therapeutic target for preventing cancer metastasis.

Researchers are now beginning to apply this type of approach to modelling pathophysiological situations of increasing complexity and clinical relevance. For instance, a microengineered 3D cell culture device has been created to establish an organ-specific

disease model that simulates complex interactions between circulating metastatic breast cancer cells, vascular endothelium and bone tissue to investigate the specificity of human breast cancer metastases to bone[33] (FIG. 2b). To reconstitute the tissue–tissue interface between the endothelium and bone, this model used a lining of human umbilical vein endothelial cells in a microfluidic channel adjacent to a 3D collagen gel that contained bone cells differentiated from human bone marrow-derived mesenchymal stem cells. Cancer metastasis in this system was examined using high-resolution real-time microscopy to measure *trans*-endothelial migration of metastatic breast cancer cells circulating in the vascular channel and their subsequent migration deeper into the bone matrix. This analysis showed that the chemokine CXCL5 (produced by bone cells) and its receptor CXCR2 (expressed by tumour cells) are major signalling mediators that govern the rate and extent of cancer cell extravasation and migration, indicating that CXCL5 and CXCR2 could be potential targets for therapeutic intervention.

As illustrated by these representative studies, the organ-on-chip microengineering approach could expand our fundamental knowledge of biological systems and enable more accurate validation of organ-specific and effective therapeutic targets. Other studies have used similar microengineering principles to investigate molecular mechanisms of immune cell–cancer cell crosstalk[34], astrocyte–neuron interactions in an enzyme-mutant model of amyotrophic lateral sclerosis[35], mitochondrial cardiomyopathy of Barth syndrome[36] and biomechanical force-induced exacerbation of drug-induced toxicities in pulmonary oedema[15].

Potential uses in drug screening

Organ-on-chip technology is being used to develop cost-effective *in vitro* models for hit-to-lead and lead optimization that can more reliably predict the efficacy, toxicity and pharmacokinetics of drug compounds in humans, as well as for novel phenotypic screening assays, as described below.

Efficacy

Poor efficacy in clinical trials is caused by an absence of predicted therapeutic effects or the unexpected need to increase the dose to levels at which toxicity also becomes apparent. In these cases, the undesirable outcome results directly from the failure of existing methods to accurately predict *in vivo* drug efficacy before clinical trials. Human organ-on-a-chip models are well suited to have an instrumental role in addressing the limitations of existing methods by providing a means to reproduce and pharmacologically modulate key aetiologies and clinically relevant integrated downstream responses at varying levels of complexity.

The potential of organ-on-chip approaches for testing drug efficacy has been explored in a recent study that developed a microengineered 3D assay of epithelial– mesenchymal transition (EMT) during cancer progression[37] (FIG. 2c). By culturing lung cancer spheroids in a 3D matrix gel adjacent to an endothelialized microchannel, this model recapitulated EMT-induced tumour dispersion and phenotypic changes in cancer cells in an endothelial cell-dependent manner. To demonstrate the potential of this system as a drug screening platform, 12 drugs — ranging from prospective drugs early in the discovery pipeline to US Food and Drug Administration (FDA)-approved drugs — were introduced to the vascular

channel, and their ability to inhibit EMT was analysed by direct visualization of the cancer spheroids. Interestingly, the effective drug concentrations in this model differed by as much as three orders of magnitude from those measured in a conventional 2D dispersion assay. Moreover, the relative rankings of the 12 drugs, as ordered by the concentration required to inhibit EMT, were not consistent with results from 2D culture. More importantly, the efficacious concentrations determined by this cancer-on-a-chip system were closer approximations of effective drug concentrations in humans measured during clinical trials than were the concentrations determined from the 2D model. This type of efficacy comparison between lead compounds and currently marketed drugs could have a profound influence on go/no-go decisions during lead optimization, as it is desirable for candidate new molecular entities to outperform other available options in some capacity. Similar discrepancies between effective drug concentrations predicted by organ-on-chip systems and those by traditional 2D assays have been demonstrated using a microengineered model of a mammary duct in breast cancer[38] and a multi-organ model of uterine cancer, the liver and bone marrow[39].

An *in vivo* tissue engineering strategy was recently used to increase the complexity and physiological relevance of organs-on-chips[40]. This study described a novel method in which a polymeric chamber containing bone-inducing materials was implanted into murine hosts to generate new bone that encased intact living bone marrow, which was then removed from the chamber and cultured in a microfluidic device[40] (FIG. 3). This bone-marrow-on-a-chip system reconstituted a complex 3D haematopoietic niche and preserved bone marrow-specific organ functionalities by maintaining the spatial distribution and physiological proportions of haematopoietic stem cells and progenitor cells *in vitro*. In this system, granulocyte colony-stimulating factor promoted bone marrow recovery after exposure to clinically relevant doses of γ-radiation, a therapeutic implication that was not evident in conventional bone marrow culture systems. By using the *in vivo* environment to precondition engineered tissue, this system retained complex physiological activities while leveraging microfluidics for sample manipulation and assessment.

Similarly, another study cultured *ex vivo* brain tissue retaining complex *in vivo* neural network connections and electrophysiological behaviour in a microsystem that permitted localized pharmacological treatment[41] (FIG. 4). Specifically, two thin brain tissue slices were enclosed in neighbouring microfluidic channels and allowed to form synaptic connections, resulting in synchronized electrophysiological burst activity. The culture medium in one microchannel was supplemented with kynurenic acid, a glutamate receptor antagonist, which selectively inhibited the spontaneous electrical excitation of the treated brain slice with no effect on the other slice. This study demonstrates the potential for integrating *ex vivo* tissue with organ-on-chip platforms to probe higher-order functionality of complex tissue with precise spatiotemporal chemical and pharmacological intervention for drug efficacy studies.

As illustrated by these studies, engineering organ-on-chip microdevices using *in vivo* environments or *ex vivo* tissues may be a viable strategy for model development and optimization, especially when conventional *in vitro* approaches are not fully capable of

replicating the organ-specific structural and functional complexity that is critical for reliable assessment of drug efficacy.

Toxicity screening

Unexpected adverse drug effects are the second most common cause of clinical trial failures and are also responsible for the costly withdrawals of marketed drugs. Established biochemical models can evaluate specific and anticipated mechanisms of toxicity (for example, hERG (also known as KCNH2) channel binding; the hERG is a potassium voltage-gated channel), and cellular models can monitor simplistic end points such as cytotoxicity. Animal testing often fails to detect important human toxicities and can cause unnecessary rejection of drug candidates based on animal-specific pathways of toxicity. Organ-on-chip technology could be used to assess human-relevant drug responses for anticipated toxicities at various levels of biological complexity (that is, the subcellular, cellular, tissue and organ levels) and to detect unanticipated off-target toxicities.

The primary focus of research in this area has been on microengineered liver models, owing to the current emphasis on testing for hepatotoxicity and the central role of hepatic drug metabolism in adverse drug reactions. The proof of principle for this approach has been demonstrated in recent studies that utilized human liver-on-a-chip devices for metabolomic analysis of hepatotoxicity. For example, microfluidic culture of human hepatocytes was coupled with NMR spectroscopy to develop a bioanalytical platform for monitoring metabolic responses of hepatocytes to the anticancer drug flutamide and its active metabolite hydroxyflutamide, both of which are known to possess hepatotoxic properties[42]. Through quantitative analysis and mapping of metabolic and mitochondrial activities, the investigators identified metabolic signatures of toxic drug responses in this model, and delineated metabolic pathways involved in the induction of hepatotoxicity due to flutamide and hydroxyflutamide. The organ-on-chip approach leveraged in this study was instrumental in reducing biological noise inherent to *in vivo* metabolomics models, and revealed a potential source of hepatotoxicity-specific biomarkers.

A similar strategy was used in creating a microengineered model that integrated microfluidic culture of human hepatocytes embedded in a 3D hydrogel with in-line mass spectrometry to study the toxicity of acetaminophen metabolites[43]. Furthermore, extension of this approach made it possible to model the secondary toxic effects of drug metabolites produced in the liver on other organs, as demonstrated by a microfluidic liver–kidney model that simulated the systemic interaction between these two organs and demonstrated nephrotoxic responses to the hepatic metabolite of ifosfamide[44]. More recently, researchers have begun to explore the potential of organ-on-chip technology beyond hepatotoxicity screening by developing microdevices that enable the reconstitution and monitoring of contractile functions of heart muscle for quantitative analysis of drug-induced cardiotoxicity[45–49].

Organ-on-chip microsystems can also be used to model clinically relevant off-target drug toxicities other than liver and heart failure. For example, we recently used a microengineered lung-on-a-chip model to simulate the pulmonary oedema caused by the dose-limiting toxicity of IL-2 that is observed in patients with cancer[15]. When the vascular microchannel lined with human microvascular endothelial cells was treated with clinically used

concentrations of IL-2, this microdevice replicated the leakage of intravascular fluid into the air-filled alveolar compartment and concomitant flooding of the air space. Intra-alveolar deposition of fibrin clots caused by the activation of coagulation cascades was also observed in this model, which is another clinically relevant toxic response that occurs during the course of IL-2-induced pulmonary oedema. High-resolution microfluorimetric analysis of the alveolar–capillary interface showed that the IL-2 toxicity-induced oedematous responses are elicited by compromised intercellular junctions and resultant increases in barrier permeability. Furthermore, this mechanically active model was capable of mimicking physiological breathing motions, and breathing-generated mechanical forces were found to increase IL-2-induced tissue injury[50]. This injury was effectively inhibited by potential drug candidates, such as angiopoietin-1 and a newly developed transient receptor potential vanilloid 4 (TRPV4) ion channel blocker[50].

Organ-on-a-chip models can therefore be used for accurate prediction and mechanistic investigation of dose-limiting human toxicities of prospective drugs, as well as for the exploration of new therapeutic approaches to mitigate the observed toxic effects. In the drug discovery pipeline, predictions made by these models could inform and facilitate early efforts to identify, modify and optimize lead compounds, thus developing safer drugs with increased likelihood of success in clinical trials[51].

Pharmacokinetics and body-on-chip systems

Several studies have demonstrated the potential of organ-on-chip microdevices to model key pharmacokinetic processes that govern drug bioavailability, with a strong emphasis on drug metabolism. Liver-on-a-chip models integrated with physiologically based pharmacokinetic computational models have been particularly useful for predicting rates of metabolic drug clearance that correlate with clinical data[52]. For instance, this integrative liver-on-a-chip approach was used to culture human primary hepatocytes in 12 parallel microfluidic chambers and to test the metabolism of 7 drugs simultaneously[53]. Detection and quantification of drug metabolites revealed that five cytochrome P450 enzymes — mediators of phase I drug metabolism — were active in these systems. Moreover, drug clearances in this microsystem predicted by a physiologically based pharmacokinetic model closely matched those in previously reported clinical studies. Similar predictive capabilities were demonstrated in another study that used microfluidic perfusion culture of primary human hepatocytes to investigate the clearance of six marketed drugs[54].

Advances in organ-on-chip technology have also made it possible to develop *in vitro* models of other critical processes that determine the bioavailability of drug compounds, such as absorption and excretion. For example, Kim and colleagues[55,56] created a 3D microfluidic system consisting of two overlapping cell culture chambers separated by a transparent elastomeric membrane lined with Caco-2 cells to model the intestinal barrier. This gut-on-a-chip system enabled prolonged culture of microbial cells on the epithelial surface in the presence of physiological luminal flow and used negative pressure-driven membrane stretching to mimic peristaltic motions to recapitulate the dynamic mechanical microenvironment of the intestine. Three-dimensional tissue microstructures that resembled intestinal villi and consisted of heterogeneous cell populations of native intestinal epithelium

formed in this environment. Importantly, this model reproduced characteristic absorptive properties and the barrier function of the human intestine, which may be particularly useful for drug absorption studies[55,56]. In addition, this system allowed for co-culture with intestinal bacteria, and so could be used to investigate the role of the gut microbiome in drug absorption. The compartmentalized channel design of this system was also used in a separate study to generate a model of the intestine–capillary interface[57]. By integrating microfluidic cell culture of Caco-2 cells on a semi-porous membrane with in-line mass spectroscopy, this model enabled real-time analysis of intestinal permeability to a model drug, curcumin, and generated data consistent with previous findings on human intestinal barrier function.

Although these individual organ models offer compelling advantages, there has been increasing recognition that the full potential of organ-on-chip technology for preclinical testing, particularly for modelling pharmacokinetics and pharmacodynamics, can only be realized by integrating these models to recapitulate human physiology at the whole-body level. Motivated by this vision, researchers have begun to pursue the development of multi-organ models capable of replicating sequential processes that influence drug activity within the body. The most common approach to creating this type of system is to link together multiple microengineered organ models via a fluidic network that allows for their functional integration and interaction in a physiologically relevant manner. Recent advances in the field have been made possible by the unique capability of these human ‘body-on-a-chip’ systems to predict body-level drug responses that reflect both complex organ-level effects and organ–organ interactions[58,59]. One such study combined models of breast cancer, the intestine and liver to establish a network of interconnected microfabricated cell culture chambers to demonstrate the sequential absorption, metabolism, and efficacy of four anticancer drugs[60] (FIG. 5a). An extension of this system incorporated a gastric acid stomach chamber to simulate gastrointestinal digestion of drugs before intestinal absorption, demonstrating that integrated human models can be developed to capture entire physiological processes that determine bioavailability and efficacy of orally administered drugs[61].

Body-on-chip strategies have also been useful in modelling the circulation and excretion of drugs. For example, an integrated model consisting of a microengineered peristaltic pump and an albumin-containing blood simulant to mimic circulating blood, a dialysis membrane to simulate excretion, and a chamber containing breast cancer cells was used to evaluate anticancer drug excretion and retention[62]. In this model, the retention and efficacy of thio-TEPA (*N,N′N′*-triethylenethiophosphoramide) and docetaxel was greatly influenced by their affinity for albumin.

The design and operation of these microengineered multi-organ models are often challenging and require careful consideration of allometric scaling principles[63]. As an organism is scaled down to a microdevice model, imbalances are inevitable between parameters that scale differently with size. Within a single organ microsystem, these include media volume, cell number, drug concentration and reaction kinetics. When multiple organ models are linked together, additional parameters such as relative organ volumes, blood flow rates, organ interaction times and drug–tissue partition coefficients become important. To predict drug performance in these models, we must anticipate scaling effects, identify

critical parameters depending on the purpose of the particular model (for example, mechanistic proof-of-principle or dose determination), and design the system to ensure that the parameters accurately reflect *in vivo* values.

To address these scaling considerations, Shuler and colleagues[64] pioneered the application of pharmacokinetic and pharmacodynamic modelling principles to develop more physiologically realistic integrated systems known as micro cell culture analogues. For example, a multi-organ model comprising interconnected microchambers representing a colon tumour, the liver and bone marrow recapitulated the *in vivo* distribution, retention and recirculation of drug-containing blood in these organs (FIG. 5b). In this model, the residence times of medium in each organ compartment were matched to blood residence times in the *in vivo* counterparts. Moreover, integration of 3D cell culture in hydrogels in this system enabled the investigation of hepatic metabolism-mediated cytotoxicity of the prodrug tegafur to colon cancer, liver cancer and bone marrow cells.

In another study, the same group demonstrated the applicability of these systems to link *in vitro* micro cell culture analogues with computational pharmacokinetic and pharmacodynamic models[65]. A microfluidic device was created to model tegafur and 5-fluorouracil anticancer activity by recirculating common media through colon cancer, liver and bone marrow compartments. Toxicity data from this study were analysed in conjunction with a computational, physiologically based pharmacokinetic and pharmacodynamic model that reflected flow rates, media volumes, drug concentrations, reaction kinetics and cytotoxic activity in the microdevice. In this analysis, experimentally measured cytotoxicity was used to refine the computational model to generate more accurate estimates of hepatocyte, colon carcinoma and haematopoetic stem cell death *in vivo*. This study demonstrates an integrative strategy that combines microengineered cell culture with a numerical pharmacokinetic and pharmacodynamic approach to address scaling allometry in organ-on-chip models and to produce human-relevant pharmacokinetic and pharmacodynamic predictions.

These studies suggest that organ-on-chip technology could be used to develop novel assays to simulate and predict critical physiological responses involved in drug bioavailability, efficacy and toxicity.

Phenotypic screening

Target-based drug discovery requires the generation and testing of specific mechanistic molecular hypotheses to identify therapeutic targets and drug candidates, whereas the newly resurgent process of phenotypic drug discovery is driven by screening of specific physiological responses in cell-based or animal models[66]. The goal of phenotypic screening is to identify active compounds that ameliorate disease phenotypes without initial concern for the molecular mechanisms of action of the identified compounds.

For effective phenotypic screening, it is critical that the assay captures various aspects of complex human physiology while remaining amenable to high-throughput manipulation for efficient appraisal of large compound libraries. However, despite recent efforts towards assay development and automation, current phenotypic models leave substantial room for improvement to meet both of these requirements. Animal assays have been productive and

serve as a more physiological approach but remain expensive, whereas cell-based assays do not generally provide valuable phenotypic data except in particular cases in which static multi-well plate cell culture can express complex behaviour (for example, angiogenesis[67]). These limitations can be addressed by the intrinsic advantages of organs-on-chips, which offer small-scale platforms with low reagent-volume requirements that are compatible with automated imaging-based analytics.

For example, Trietsch *et al.*[68] developed a microengineered high-throughput 3D cell culture platform that modified a conventional 384-well plate to create as many as 96 independent organ-on-chip microchambers. Each chamber contained three interconnected parallel microchannels that enabled the formation and continuous perfusion of cell-laden hydrogels. This microdevice was used to co-culture human hepatocytes with fibroblasts to assess a concentration-dependent hepatotoxic response to rifampicin. Hepatocyte viability decreased when rifampicin concentrations exceeded 160 μM, and this toxic response became more pronounced with longer exposure times. Similar platforms, with self-assembled 3D tissues in microengineered matrices, were used to create an array of contractile cardiac microtissues that permitted simulation of arrhythmia and higher-throughput screening of phenotypic changes[48]. The cardiac microtissues in this model exhibited a high-frequency activation rate with no rest period, similar to an *in vivo* reentrant state of arrhythmia. Moreover, an electrical defibrillation stimulus could revert this phenotype to a normal rhythm. These types of organ-on-chip models are constructed in conventional multi-well plates and are therefore readily scalable and fully compatible with standard liquid handling and analytical equipment, which is important for higher-throughput phenotypic screening.

Phenotypic screening can also be used to investigate approved drugs or drugs that failed to meet their primary efficacy end points in Phase III clinical trials, and repurpose them for the treatment of other diseases. The majority of successfully repositioned drugs have been identified through serendipitous findings in animal studies, clinical trials and clinical practice, all of which are expensive. It has therefore been suggested that phenotypic screening using predictive preclinical models may be the most efficient and cost-effective way to reposition drugs[1,69,70]. Researchers have already begun to explore the application of organs-on-chips as predictive phenotypic assay platforms for drug repositioning. For example, a microengineered model of liver cancer was used to investigate the anticancer effects of artemisinin, an antimalarial drug that is currently being considered for use as a cancer therapy[71]. In this model, human liver cancer cells self-assembled into 3D tumour structures and their invasive migration into neighbouring micropatterned 3D gel matrices was examined under physiological interstitial flow. Microfluidic delivery of artemisinin to the solid tumours led to increased cell–cell adhesion and effective inhibition of cancer cell migration, thus demonstrating the potential of the drug as an anticancer therapeutic. When combined with the continuing progress in creating microengineered multi-organ models of integrated whole body physiology, this organ-on-chip-based phenotypic screening approach might greatly facilitate the increasing efforts to harness the potential of drug repositioning.

Further applications

In addition to improving research and development efficiency in general, organ-on-chip platforms could be useful to support and accelerate efforts in rare diseases, stratified medicine and nanomedicine, fields that are attracting increasing industrial interest. Exploring these further applications could be facilitated by the integration of organs-on-chips with other emerging disease modelling techniques, such as sourcing cells from patients to develop induced pluripotent stem cells (iPS cells), or modifying normal stem cells with new gene editing tools (for example, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system).

Rare diseases

Rare diseases have attracted growing commercial interest in recent years[72]. However, the development of new drugs for these diseases is greatly hampered by the lack of appropriate preclinical models and the scarcity of patient populations available for clinical trials; therefore, organ-on-chip technology could have a role in developing better models. For example, a heart-on-a-chip model incorporating genetically engineered iPS cells revealed that contractile weakness of cardiomyocytes in Barth syndrome is caused by a mutation in the gene encoding tafazzin, thus providing insight into key disease mechanisms that may reveal potential therapeutic targets for this rare disease[36]. In a similar attempt to model hereditary haemorrhagic telangiectasia, van der Meer and colleagues[73] utilized a microfluidic device to engineer 3D vascular tissue constructs into blood vessel-like tubular structures. Interestingly, inhibition of transforming growth factor-β signalling pathways in this model resulted in the formation of disorganized and tortuous vascular tubes that closely resembled the abnormal blood vessels characteristic of the disease. This model may provide a basis for further investigation of the biological underpinnings of haemorrhagic telangiectasia.

Stratified medicine

The practice of developing drugs for particular patient populations — for example, stratified on the basis of the presence of a particular biomarker linked to disease or drug response — has become increasingly prevalent in recent years. One path to population-specific drugs is through post-hoc analysis of large clinical trials, but this approach is burdened by the need for enlarged and therefore more expensive clinical trials to allow analysis of statistically significant differences even between small subgroups[74]. As highlighted in the “Enrichment Strategies for Clinical Trials” recently published by the FDA[75], this problem is being tackled by the prospective use of specific patient characteristics to select subpopulations that are more likely to show desired drug responses. Clearly, preclinical models that enable these types of predictive studies earlier in the pipeline could help refine patient pools for clinical trials.

To this end, organ-on-chip technology has been applied to develop *in vitro* models that reflect genetic underpinnings of variability in human drug responses. For example, a two-organ model was constructed in a microdevice consisting of two interconnected microchambers that supported 3D culture of brain tumour and liver cells[76]. By incorporating

liver cells expressing varying levels of cytochrome P450 subtypes, a set of metabolic enzymes with significant ethnic variation[77], this study examined differential responses of tumour cells to the anticancer drug ifosfamide. Activation of ifosfamide by the liver cells and resultant cytotoxic effects on the tumour cells were found to depend heavily on the level of CYP3A4 expression in liver cells. The combination of organs-on-chips and iPS cells may further provide a relatively inexpensive method to generate patient- and population-specific model platforms that could be used to test drugs that target specific mutations or polymorphisms.

Another recent development in this area is to perform complementary animal studies in parallel with human trials[78]. These co-clinical trials are designed to reflect the relevant genetic diversity of the human patient population by using cells derived from the patients in the trial to generate xenograft models, with the goals of predicting patient responses and studying the factors that contribute to outcome variability. For instance, co-clinical studies could identify genetic biomarkers that are predictive of therapeutic outcomes, and these biomarkers could be immediately validated in patients. Organs-on-chips could provide genetically matched platforms for early prediction and mechanistic investigation of patient response or resistance to therapy, biomarker identification and screening of panels of possible therapy adjustments in the event of drug resistance or relapse. For future personalized medicine and co-clinical trial applications, a patient's own cells could be adapted with iPS cell techniques to develop patient- and population-specific organs-on-chips for truly individualized study of drug safety and efficacy or drug–drug interactions.

Nanomedicine

Recent growth in nanotechnology has fuelled intense efforts to apply research in this area to the development of therapeutics. Examples of promising approaches include the use of nanoparticles for delivery and localization of drugs to specific tissues of interest and for packaging of pharmaceuticals that would otherwise have limited bioavailability[79,80]. However, the novelty of this approach raises concerns regarding the potential adverse effects of the nanomaterials involved. So, the development, optimization and clinical translation of nanomedicines require rigorous preclinical assessment of drug-induced toxicity as well as efficacy, but these studies are challenging because of the lack of test platforms that incorporate sufficient human-relevant physiological complexity for reliable prediction of drug effects.

To provide proof of principle for using organ-on-chip approaches to meet the needs for inexpensive, predictive models in nanomedicine, Kim *et al.*[81] used a blood vessel-on-a-chip to study the translocation of lipid–polymer hybrid nanoparticles across a microfluidic endothelium mimicking the dysfunctional endothelial barrier of atherosclerosis. Importantly, key findings from this microengineered *in vitro* model were corroborated by data from animal studies using a rabbit model of atherosclerosis[81]. Similar approaches could make it possible to model and study transport of nanotherapeutics across other critical barriers in the body such as the gut[55,56] and the blood–brain barrier[82–85]. Furthermore, extension of this strategy based on integrated multi-organ models could be instrumental in evaluating

functional sustainability and bioavailability of nanotherapeutic agents such as nanoengineered RNA interference therapies[86].

Current challenges and limitations

Realizing the full potential of organ-on-chip technology comes with technical and entrepreneurial challenges. One of the critical technical challenges arises from materials such as poly(dimethylsiloxane) (PDMS) that have gained widespread use in rapid-prototyping of organ-on-chip microdevices. Existing organ-on-chip models rely heavily on cell culture substrates made of synthetic materials (for example, PDMS, polycarbonate and polyester), the physicochemical properties of which are not appropriate for mimicking extracellular matrices *in vivo*, and new cell culture substrates are needed[87]. For example, PDMS has been shown to absorb small hydrophobic molecules[20,88], which may lead to reduced effective drug concentrations and pharmacological activities. Researchers have already begun to address this problem by using chemical surface modification[89] or alternative materials[90,91]. Challenges associated with standardizing and automating the highly variable current PDMS fabrication techniques originally developed for laboratory prototyping also pose major technical hurdles to large-scale manufacturing and higher-throughput operation of organ chips, which are important steps for industrial adoption of this technology. Successful translation of organs-on-chips from proof of concept in the laboratory to commercial screening platforms will require the identification and optimization of new low-cost materials and fabrication strategies suitable for their mass production and integration into existing infrastructures in the pharmaceutical industry.

More reliable and sustainable sources of human cells, especially disease-specific cells, that are amenable to *in vitro* culture in organs-on-chips and phenotypically represent their *in vivo* counterparts are also needed. Although questions remain regarding the phenotypic maturity of their differentiated progeny, human embryonic stem cells and iPS cells have the potential to serve as cell sources that can be engineered to suit specific needs in the development of organs-on-chips[92,93]. This approach is particularly attractive given the capability of organ-on-chip models to generate and control physiologically relevant structural, biochemical and mechanical cues that instruct stem cell differentiation and maturation.

For the analysis of biological responses, the low culture volumes and cell numbers in organs-on-chips often give rise to technical issues associated with detection sensitivity. This challenge may be met by leveraging advances in lab-on-a-chip technologies[94] to integrate organ-on-chip models with bioanalytical platforms microengineered with miniaturized optics and sensors that enable high-resolution biochemical analysis with substantially reduced sample volume requirements. In addition, for effective model validation, the readouts of organs-on-chips must map to the standard clinical end points they are designed to model. This type of correlation will better enable translation and interpretation of results from organs-on-chips. However, mapping organ-on-chip readouts to their clinical counterparts is currently difficult owing to major differences in measurement techniques[49]. Computational modelling will be necessary to correlate the more simplified readouts of organs-on-chips to their corresponding clinical diagnostics and to provide context for translation to a patient response.

Another overarching consideration in organ-on-a-chip development is the balance between complexity and practicality. Increasing the complexity of an organ-on-a-chip model is required for improving its physiological relevance; however, this often presents major challenges to practical operation and management of the system. Given the capability of organs-on-chips for precise control of cell culture parameters, this balance may be achieved by identifying the minimal subset of cells and microenvironmental factors necessary to create the simplest model possible that recapitulates physiological responses of interest. If complexity is inevitable, however, the practicality of organs-on-chips can be improved by developing automated instrumentation that enables the following capabilities: physiological integration, maintenance and monitoring of microengineered cell culture models; control of cellular exposure to drug compounds with high spatiotemporal precision; and sample collection and real-time analysis of drug responses. These capabilities will be particularly useful to examine chronic disease progression and long-term drug effects[95,96], which require long-term culture and are currently a major challenge. Animal models are currently better suited to address these questions.

Improved automated instrumentation will also facilitate the development of body-on-chip models by providing independent, simultaneous control of different cell types within a single device. The success of these integrated models, however, will still rest on the development of a common culture media that can support various types of cells and mediate their biochemical communication. Formulation of such media will require a fundamental understanding and careful consideration of cell type-dependent responses to various soluble cues such as serum, growth factors and hormones. Alternatively, microengineered systems could be developed that utilize conventional multi-well plates for parallel microfluidic cell culture and replace external flow pumps with standardized robotic liquid handling systems[68,97].

Organs-on-chips are not universal solutions, and alternative tools will continue to be better solutions for modelling certain *in vivo* processes. *In vitro* models recreating the complex biology of the endocrine, immune and nervous systems remain beyond the capabilities of organs-on-chips. Use of *ex vivo* tissue as described in the ‘Efficacy’ section above could eventually be used to study these systems, but predicting clinically relevant drug effects will continue to require the use of animal models for some time. Multi-organ-on-chip systems have been used for toxicity testing; however, these studies have not been used for broad, systematic off-target toxicity, which remains a task more appropriate for animal testing or adverse outcome pathways analysis. Also, although several organs-on-chips have been used for pharmacological dose-finding studies (as discussed in the ‘Pharmacokinetics and body-on-chip systems’ section), computational pharmacokinetic modelling based on physicochemical drug properties and estimations of metabolism is a lower-cost alternative for early preclinical estimates of bioavailability, and animal testing continues to be a more complete predictive tool for later testing.

In the many cases in which organs-on-chips offer improved biological modelling, alternative methods may still be preferable for practical considerations. In target identification and validation, efficacy testing and toxicity testing, it is not always critical to recapitulate integrated, organ-level functionality, and in these cases more cost-effective biochemical and

cellular assays can be effectively implemented. It is also important to recognize that organ-on-chip model development will take a substantial amount of time to become suitable for standard use throughout the drug discovery process, whereas existing biochemical, cellular and animal models offer off-the-shelf solutions validated for regulatory acceptance.

Although many of the above challenges may be addressed by progress in the organ-on-a-chip field, there are also intrinsic limitations that organs-on-chips may never circumvent. These model platforms necessarily include only a subset of the entire milieu of cell types, tissues, organs and systems present *in vivo*, and therefore will not detect every possible off-target toxicity. Animal tests instead offer whole-organism toxicity testing, so there may be persistent value for supplementing organs-on-chips with whole-animal studies. For reductionist investigations throughout the drug discovery and development process (for example, water solubility for bioavailability and interrogation of mechanism-of-action of identified toxicities), organs-on-chips may include irrelevant complexity, resulting in unnecessary costs and measurement variability compared to biochemical and cellular assays or *in silico* analyses. Similarly, organ-on-chip testing will be more costly than biochemical and cellular testing in high-throughput contexts. For screening a large compound library for target activity, it is unlikely that organs-on-chips will improve accuracy enough to justify their added costs over traditional high-throughput biochemical and cellular assays.

Opportunities to be explored

From a technological standpoint, extending the current state-of-the-art in organ-on-chip technology will provide new opportunities to model several organs that have not yet been fully explored in this field. Of particular interest are those in the nervous, endocrine, sensory, digestive and reproductive systems that are often a target of therapeutic intervention and/or affect the pharmacological activity of drugs. Success in this endeavour will require the formation of alliances between engineers, biologists, computational biologists and clinicians to devise new methodologies for capturing the essential components of the physiology of target organs.

Organ-on-chip technology can be synergistically integrated with modelling and analysis tools already used in drug discovery. Many validation technologies are designed predominantly for molecular-level analysis of therapeutic targets, but can be complemented by organs-on-chips to predict organ-level responses. For example, organ-on-chip models could be used to validate potential targets identified by genomics and proteomics analyses. A similar synergy can be achieved in the later steps of the drug discovery process. For instance, organs-on-chips may serve as a complementary tool in adverse outcomes pathway analysis[98] to detect novel toxicities that are not covered by established libraries of known toxic interactions. For the development of specialized therapeutics such as medical countermeasures against radiological, chemical or biological agents for which it is unethical to conduct human clinical trials, organs-on-chips may augment animal studies conducted under the FDA's Animal Rule[99].

Organs-on-chips could also be used to model drug delivery and transport through complex 3D tissues (for example, solid tumours). This is an important consideration for

pharmacodynamic analyses to determine or validate drug doses and administration regimens required to achieve desired therapeutic effects. In addition, sampling and analysis of conditioned medium from these physiological 3D models could be used to identify or validate predictive biomarkers of drug efficacy and toxicity.

Another important area of opportunity for future studies is the application of organ-on-chip technology for the development of predictive human disease models. For example, one area of potential interest is to use organs-on-chips to model paediatric diseases and rare diseases, studies of which are limited due to the paucity of *in vitro* modelling approaches, small patient populations and limited patient availability. Reliable cell sources will be important for organs-on-chips to contribute to the development of predictive human paediatric disease models.

Perhaps even more critical for the progression and maturation of this field is to develop robust strategies to recapitulate the functional integration of multiple organs and to simulate human physiology and pathology at the whole-body level. The most promising prospective application of body-on-a-chip (as well as organ-specific) systems is to bridge the large gap between preclinical predictions based on animal studies and the actual outcomes of clinical trials[100]. In the near term, organ-on-chip technology may serve as a complementary preclinical approach that could support or disprove predictions from animal testing before clinical studies.

Outlook

Despite their limitations, organs-on-chips have the potential to play a transformative role across drug discovery and development. Rigorous validation of this technology using not only animal data but, more importantly, the results of clinical trials is required to determine whether organ-on-chip models accurately represent human-relevant physiology and show predictive capability across broad drug classes and clinical outcomes.

Eventually, organ-on-a-chip models may play a pivotal role in streamlining the clinical trial process. For example, advances in stem cell engineering could be integrated into organ-on-chip technology to develop personalized models to predict patient-specific toxicity and efficacy, which could lead to more efficient human trials with significantly reduced preclinical testing requirements. These personalized models may also be useful in exploring patient-specific biomarkers and individualized dosing regimens based on patient-specific pharmacokinetics[101]. In a related strategy, organ-on-chip models could be used to explore broad population genetic variations that could reduce the required size of clinical trials.

Ultimately, the organ-on-a-chip field will only achieve its full potential with translation into commercial drug discovery. This undertaking requires that organ-on-a-chip models become packaged test solutions acceptable to the pharmaceutical industry: assays must be reliable and robust, and not require specialized personnel with unique technical expertise. Regulatory agencies are understandably circumspect in accepting new sources of data as justification for human trials, therefore organ-on-chip models will also have to survive stringent validation to serve as formal preclinical tests. Achieving the necessary levels of development and validation for commercial marketability is, at its heart, an entrepreneurial challenge. Such

challenges include the requirement of start-up funding for technical development, industry and regulatory acceptance, and historical validation of data, all of which constitute a multidimensional chicken-or-egg dilemma, as each necessary component is difficult to achieve without prior attainment of the other components. However, these hurdles could be surmountable with tri-lateral partnerships between academic institutions, industry and regulatory agencies. As illustrated by the recent launch of US federal funding programmes for integrated microphysiological systems[102,103], government and regulatory agencies have recognized the paradigm-shifting potential of organ-on-chip technology. Pharmaceutical companies are also beginning to establish partnerships with leading academic institutions to jointly explore this technology and to position themselves at the forefront of expected organ-on-a-chip advances.

Achievements in the organ-on-a-chip field could present exciting new avenues for drug discovery and development. Much remains to be done, and accordingly there is a wealth of opportunities to participate in what we hope will be a surge of innovation to realize the tremendous potential that organ-on-chip technology holds.

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Glossary

Biomimetic	Refers to the use of principles, mechanisms and designs derived from those naturally occurring in living organisms
Epithelial–mesenchymal transition	(EMT). The process by which a polarized epithelial cell undergoes a series of biochemical changes to acquire characteristics of a mesenchymal cell, including increased invasive and migratory capacity, higher resistance to apoptosis and upregulated production of extracellular matrix proteins
Microfluidics	A science and engineering discipline focusing on the development of fluidic systems with characteristic dimensions of tens to hundreds of micrometres that provide capabilities to control, manipulate and analyse small volumes of fluids (microlitres to attolitres) for a wide range of applications
Spheroids	Three-dimensional spherical agglomerations of adherent cells generated by intercellular adhesion and aggregation
Stratified medicine	An approach that aims to develop patient-specific therapies using biological or risk characteristics (for example, biomarkers and genetics) shared by subgroups of patient populations. This approach is also referred to as personalized or precision medicine

References

1. Scannell JW, Blanckley A, Boldon H, Warrington B. Diagnosing the decline in pharmaceutical R&D efficiency. Nature Rev Drug Discov. 2012; 11:191–200. [PubMed: 22378269]
2. Paul SM, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature Rev Drug Discov. 2010; 9:203–214. [PubMed: 20168317]
3. Caponigro G, Sellers WR. Advances in the preclinical testing of cancer therapeutic hypotheses. Nature Rev Drug Discov. 2011; 10:179–187. [PubMed: 21358737]
4. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nature Rev Drug Discov. 2004; 3:711–715. [PubMed: 15286737]
5. Bowes J, et al. Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling. Nature Rev Drug Discov. 2012; 11:909–922. [PubMed: 23197038]
6. Muller PY, Milton MN. The determination and interpretation of the therapeutic index in drug development. Nature Rev Drug Discov. 2012; 11:751–761. [PubMed: 22935759]
7. Folch A, Toner M. Microengineering of cellular interactions. Annu Rev Biomed Eng. 2000; 2:227–256. [PubMed: 11701512]
8. Whitesides GM, Ostuni E, Takayama S, Jiang XY, Ingber DE. Soft lithography in biology and biochemistry. Annu Rev Biomed Eng. 2001; 3:335–373. [PubMed: 11447067]
9. Beebe DJ, Mensing GA, Walker GM. Physics and applications of microfluidics in biology. Annu Rev Biomed Eng. 2002; 4:261–286. [PubMed: 12117759]
10. Shamir ER, Ewald AJ. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nature Rev Mol Cell Biol. 2014; 15:647–664. [PubMed: 25237826]
11. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. Trends Cell Biol. 2011; 21:745–754. [PubMed: 22033488]
12. Yum K, Hong SG, Healy KE, Lee LP. Physiologically relevant organs on chips. Biotechnol J. 2014; 9:16–27. [PubMed: 24357624]
13. Huh D, et al. Reconstituting organ-level lung functions on a chip. Science. 2010; 328:1662–1668. [PubMed: 20576885]
14. Inamdar NK, Borenstein JT. Microfluidic cell culture models for tissue engineering. Curr Opin Biotechnol. 2011; 22:681–689. [PubMed: 21723720]
15. Huh D, et al. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med. 2012; 4:159ra147.
16. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. Nature Biotech. 2007; 26:120–126.
17. Bhatia SN, Balis UJ, Yarmush ML, Toner M. Effect of cell–cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and non-parenchymal cells. FASEB J. 1999; 13:1883–1900. [PubMed: 10544172]
18. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE. Microengineered physiological biomimicry: organs-on-chips. Lab Chip. 2012; 12:2156–2164. [PubMed: 22555377]
19. Ghaemmaghami AM, Hancock MJ, Harrington H, Kaji H, Khademhosseini A. Biomimetic tissues on a chip for drug discovery. Drug Discov Today. 2012; 17:173–181. [PubMed: 22094245]
20. van der Meer AD, van den Berg A. Organs-on-chips: breaking the *in vitro* impasse. Integr Biol (Camb). 2012; 4:461–470. [PubMed: 22388577]
21. Griffith LG, Swartz MA. Capturing complex 3D tissue physiology *in vitro*. Nature Rev Mol Cell Biol. 2006; 7:211–224. [PubMed: 16496023]
22. El-Ali J, Sorger PK, Jensen KF. Cells on chips. Nature. 2006; 442:403–411. [PubMed: 16871208]
23. Whitesides GM. The origins and the future of microfluidics. Nature. 2006; 442:368–373. [PubMed: 16871203]
24. Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. Nature. 2014; 507:181–189. [PubMed: 24622198]
25. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nature Biotech. 2014; 32:760–772.

26. Olson H, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharm. 2000; 32:56–67.
27. Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res. 2014; 6:114–118. [PubMed: 24489990]
28. Seok J, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci USA. 2013; 110:3507–3512. [PubMed: 23401516]
29. Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM, Hackam DG. Threats to validity in the design and conduct of preclinical efficacy studies: a systematic review of guidelines for *in vivo* animal experiments. PLoS Med. 2013; 10:e1001489. [PubMed: 23935460]
30. Li F, Yin Z, Jin G, Zhao H, Wong ST. Chapter 17: bioimage informatics for systems pharmacology. PLoS Comput Biol. 2013; 9:e1003043. [PubMed: 23633943]
31. Polini A, et al. Organs-on-a-chip: a new tool for drug discovery. Expert Opin Drug Dis. 2014; 9:335–352.
32. Song JW, et al. Microfluidic endothelium for studying the intravascular adhesion of metastatic breast cancer cells. PLoS ONE. 2009; 4:e5756. [PubMed: 19484126]
33. Bersini S, et al. A microfluidic 3D *in vitro* model for specificity of breast cancer metastasis to bone. Biomaterials. 2014; 35:2454–2461. [PubMed: 24388382]
34. Businaro L, et al. Cross talk between cancer and immune cells: exploring complex dynamics in a microfluidic environment. Lab Chip. 2013; 13:229–239. [PubMed: 23108434]
35. Kunze A, et al. Astrocyte–neuron co-culture on microchips based on the model of SOD mutation to mimic ALS. Integr Biol (Camb). 2013; 5:964–975. [PubMed: 23695230]
36. Wang G, et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nature Med. 2014; 20:616–623. [PubMed: 24813252]
37. Aref AR, et al. Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. Integr Biol (Camb). 2013; 5:381–389. [PubMed: 23172153]
38. Vidi PA, et al. Disease-on-a-chip: mimicry of tumor growth in mammary ducts. Lab Chip. 2014; 14:172–177. [PubMed: 24202525]
39. Tatosian DA, Shuler ML. A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. Biotechnol Bioeng. 2009; 103:187–198. [PubMed: 19137589]
40. Torisawa YS, et al. Bone marrow-on-a-chip replicates hematopoietic niche physiology *in vitro*. Nature Methods. 2014; 11:663–669. [PubMed: 24793454]
41. Berdichevsky Y, Staley KJ, Yarmush ML. Building and manipulating neural pathways with microfluidics. Lab Chip. 2010; 10:999–1004. [PubMed: 20358106]
42. Snouber LC, et al. Metabolomics-on-a-chip of hepatotoxicity induced by anticancer drug flutamide and its active metabolite hydroxyflutamide using HepG2/C3a microfluidic biochips. Toxicol Sci. 2013; 132:8–20. [PubMed: 22843567]
43. Mao S, Gao D, Liu W, Wei H, Lin J-M. Imitation of drug metabolism in human liver and cytotoxicity assay using a microfluidic device coupled to mass spectrometric detection. Lab Chip. 2012; 12:219–226. [PubMed: 22094544]
44. Choucha-Snouber L, et al. Investigation of ifosfamide nephrotoxicity induced in a liver–kidney co-culture biochip. Biotechnol Bioeng. 2013; 110:597–608. [PubMed: 22887128]
45. Agarwal A, Goss JA, Cho A, McCain ML, Parker KK. Microfluidic heart on a chip for higher throughput pharmacological studies. Lab Chip. 2013; 13:3599–3608. [PubMed: 23807141]
46. Grosberg A, Alford PW, McCain ML, Parker KK. Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip. Lab Chip. 2011; 11:4165–4173. [PubMed: 22072288]
47. McCain ML, Sheehy SP, Grosberg A, Goss JA, Parker KK. Recapitulating maladaptive, multiscale remodeling of failing myocardium on a chip. Proc Natl Acad Sci USA. 2013; 110:9770–9775. [PubMed: 23716679]
48. Thavandiran N, et al. Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. Proc Natl Acad Sci USA. 2013; 110:E4698–E4707. [PubMed: 24255110]

49. Capulli AK, et al. Approaching the *in vitro* clinical trial: engineering organs on chips. Lab Chip. 2014; 14:3181–3186. [PubMed: 24828385]
50. Thorneloe KS, et al. An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure. Sci Transl Med. 2012; 4:159ra148.
51. Kramer JA, Sagartz JE, Morris DL. The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. Nature Rev Drug Discov. 2007; 6:636–649. [PubMed: 17643090]
52. LeCluyse EL, Witek RP, Andersen ME, Powers MJ. Organotypic liver culture models: meeting current challenges in toxicity testing. Crit Rev Toxicol. 2012; 42:501–548. [PubMed: 22582993]
53. Baudoin R, et al. Evaluation of seven drug metabolisms and clearances by cryopreserved human primary hepatocytes cultivated in microfluidic biochips. Xenobiotica. 2013; 43:140–152. [PubMed: 22830982]
54. Chao P, Maguire T, Novik E, Cheng K-C, Yarmush M. Evaluation of a microfluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human. Biochem Pharmacol. 2009; 78:625–632. [PubMed: 19463793]
55. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip. 2012; 12:2165–2174. [PubMed: 22434367]
56. Kim HJ, Ingber DE. Gut-on-a-chip microenvironment induces human intestinal cells to undergo villus differentiation. Integr Biol (Camb). 2013; 5:1130–1140. [PubMed: 23817533]
57. Gao D, Liu H, Lin JM, Wang Y, Jiang Y. Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device. Lab Chip. 2013; 13:978–985. [PubMed: 23340920]
58. Lee JB, Sung JH. Organ-on-a-chip technology and microfluidic whole-body models for pharmacokinetic drug toxicity screening. Biotechnol J. 2013; 8:1258–1266. [PubMed: 24038956]
59. Sung JH, et al. Microfabricated mammalian organ systems and their integration into models of whole animals and humans. Lab Chip. 2013; 13:1201–1212. [PubMed: 23388858]
60. Imura Y, Sato K, Yoshimura E. Micro total bioassay system for ingested substances: assessment of intestinal absorption, hepatic metabolism, and bioactivity. Anal Chem. 2010; 82:9983–9988. [PubMed: 21090751]
61. Imura Y, Yoshimura E, Sato K. Micro total bioassay system for oral drugs: evaluation of gastrointestinal degradation, intestinal absorption, hepatic metabolism, and bioactivity. Anal Sci. 2012; 28:197–199. [PubMed: 22451356]
62. Imura Y, Yoshimura E, Sato K. Microcirculation system with a dialysis part for bioassays evaluating anticancer activity and retention. Anal Chem. 2013; 85:1683–1688. [PubMed: 23249269]
63. Wikswo JP, et al. Scaling and systems biology for integrating multiple organs-on-a-chip. Lab Chip. 2013; 13:3496–3511. [PubMed: 23828456]
64. Sung JH, Shuler ML. A micro cell culture analog (μCCA) with 3D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anticancer drugs. Lab Chip. 2009; 9:1385–1394. [PubMed: 19417905]
65. Sung JH, Kam C, Shuler ML. A microfluidic device for a pharmacokinetic–pharmacodynamic (PK–PD) model on a chip. Lab Chip. 2010; 10:446–455. [PubMed: 20126684]
66. Zheng W, Thorne N, McKew JC. Phenotypic screens as a renewed approach for drug discovery. Drug Discov Today. 2013; 18:1067–1073. [PubMed: 23850704]
67. Lee JA, Uhlik MT, Moxham CM, Tomandl D, Sall DJ. Modern phenotypic drug discovery is a viable, neoclassic pharma strategy. J Med Chem. 2012; 55:4527–4538. [PubMed: 22409666]
68. Trietsch SJ, Israels GD, Joore J, Hankemeier T, Vulto P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip. 2013; 13:3548–3554. [PubMed: 23887749]
69. Demonaco HJ, Ali A, Hippel E. The major role of clinicians in the discovery of off-label drug therapies. Pharmacotherapy. 2006; 26:323–332. [PubMed: 16503712]
70. Swinney DC, Anthony J. How were new medicines discovered? Nature Rev Drug Discov. 2011; 10:507–519. [PubMed: 21701501]

71. Kalchman J, et al. A three-dimensional microfluidic tumor cell migration assay to screen the effect of anti-migratory drugs and interstitial flow. Microfluid Nanofluid. 2013; 14:969–981.
72. Melnikova I. Rare diseases and orphan drugs. Nature Rev Drug Discov. 2012; 11:267–268. [PubMed: 22460117]
73. van der Meer AD, Orlova VV, ten Dijke P, van den Berg A, Mummery CL. Three-dimensional co-cultures of human endothelial cells and embryonic stem cell-derived pericytes inside a microfluidic device. Lab Chip. 2013; 13:3562–3568. [PubMed: 23702711]
74. Phan VH, et al. An update on ethnic differences in drug metabolism and toxicity from anti-cancer drugs. Expert Opin Drug Metab Toxicol. 2011; 7:1395–1410. [PubMed: 21950349]
75. US Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research & Center for Devices and Radiological Health. Guidance for Industry: Enrichment Strategies for Clinical Trials to Support Approval of Human Drugs and Biological Products. US: FDA; 2012.
76. Ma L, et al. Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system. Biomaterials. 2012; 33:4353–4361. [PubMed: 22429982]
77. Polimanti R, Piacentini S, Manfellotto D, Fuciarelli M. Human genetic variation of CYP450 superfamily: analysis of functional diversity in worldwide populations. Pharmacogenomics. 2012; 13:1951–1960. [PubMed: 23215887]
78. Chen Z, et al. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. Nature. 2012; 483:613–617. [PubMed: 22425996]
79. Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev. 2006; 58:1532–1555. [PubMed: 17092599]
80. Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev. 2009; 38:1759–1782. [PubMed: 19587967]
81. Kim Y, et al. Probing nanoparticle translocation across the permeable endothelium in experimental atherosclerosis. Proc Natl Acad Sci USA. 2014; 111:1078–1083. [PubMed: 24395808]
82. Prabhakarpandian B, et al. SyM-BBB: a microfluidic blood brain barrier model. Lab Chip. 2013; 13:1093–1101. [PubMed: 23344641]
83. Griep LM, et al. BBB on chip: microfluidic platform to mechanically and biochemically modulate blood– brain barrier function. Biomed Microdevices. 2013; 15:145–150. [PubMed: 22955726]
84. Achyuta AKH, et al. A modular approach to create a neurovascular unit-on-a-chip. Lab Chip. 2013; 13:542–553. [PubMed: 23108480]
85. Booth R, Kim H. Characterization of a microfluidic *in vitro* model of the blood–brain barrier (mu BBB). Lab Chip. 2012; 12:1784–1792. [PubMed: 22422217]
86. Nelson CE, et al. Balancing cationic and hydrophobic content of PEGylated siRNA polyplexes enhances endosome escape, stability, blood circulation time, and bioactivity *in vivo*. ACS Nano. 2013; 7:8870–8880. [PubMed: 24041122]
87. Flaim CJ, Chien S, Bhatia SN. An extracellular matrix microarray for probing cellular differentiation. Nature Methods. 2005; 2:119–125. [PubMed: 15782209]
88. Berthier E, Young EW, Beebe D. Engineers are from PDMS-land, biologists are from polystyrenia. Lab Chip. 2012; 12:1224–1237. [PubMed: 22318426]
89. Wong I, Ho C-M. Surface molecular property modifications for poly (dimethylsiloxane) (PDMS) based microfluidic devices. Microfluid Nanofluid. 2009; 7:291–306. [PubMed: 20357909]
90. Domansky K, et al. Clear castable polyurethane elastomer for fabrication of microfluidic devices. Lab Chip. 2013; 13:3956–3964. [PubMed: 23954953]
91. van Midwoud PM, Janse A, Merema MT, Groothuis GM, Verpoorte E. Comparison of biocompatibility and adsorption properties of different plastics for advanced microfluidic cell and tissue culture models. Anal Chem. 2012; 84:3938–3944. [PubMed: 22444457]
92. Ghafar-Zadeh E, Waldeisen JR, Lee LP. Engineered approaches to the stem cell microenvironment for cardiac tissue regeneration. Lab Chip. 2011; 11:3031–3048. [PubMed: 21785806]
93. Mathur A, et al. Human induced pluripotent stem cell-based microphysiological tissue models of myocardium and liver for drug development. Stem Cell Res Ther. 2013; 4(Suppl. 1):S14. [PubMed: 24565415]

94. Neuzil P, Giselbrecht S, Lange K, Huang TJ, Manz A. Revisiting lab-on-a-chip technology for drug discovery. Nature Rev Drug Discov. 2012; 11:620–632. [PubMed: 22850786]

95. Messner S, Agarkova I, Moritz W, Kelm J. Multi-cell type human liver microtissues for hepatotoxicity testing. Arch Toxicol. 2013; 87:209–213. [PubMed: 23143619]

96. DesRochers TM, Suter L, Roth A, Kaplan DL. Bioengineered 3D human kidney tissue, a platform for the determination of nephrotoxicity. PLoS ONE. 2013; 8:e59219. [PubMed: 23516613]

97. Meyvantsson I, Warrick JW, Hayes S, Skoien A, Beebe DJ. Automated cell culture in high density tubeless microfluidic device arrays. Lab Chip. 2008; 8:717–724. [PubMed: 18432341]

98. Bouhifd M, et al. Mapping the human toxome by systems toxicology. Basic Clin Pharmacol Toxicol. 2014; 115:24–31. [PubMed: 24443875]

99. US Food and Drug Administration, Center for Drug Evaluation and Research & Center for Biologics Evaluation and Research. Guidance for Industry: Product Development Under the Animal Rule. US: FDA; 2014.

100. Esch M, King T, Shuler M. The role of body-on-a-chip devices in drug and toxicity studies. Annu Rev Biomed Eng. 2011; 13:55–72. [PubMed: 21513459]

101. Williamson A, Singh S, Fernekorn U, Schober A. The future of the patient-specific body-on-a-chip. Lab Chip. 2013; 13:3471–3480. [PubMed: 23685915]

102. Sutherland ML, Fabre KM, Tagle DA. The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline. Stem Cell Res Ther. 2013; 4(Suppl. 1):I1. [PubMed: 24565163]

103. Dambach DM, Uppal H. Improving risk assessment. Sci Transl Med. 2012; 4:159ps22.

104. Jeon JS, et al. Generation of 3D functional microvascular networks with human mesenchymal stem cells in microfluidic systems. Integr Biol (Camb). 2014; 6:555–563. [PubMed: 24676392]

105. Lee H, Kim S, Chung M, Kim JH, Jeon NL. A bioengineered array of 3D microvessels for vascular permeability assay. Microvasc Res. 2014; 91:90–98. [PubMed: 24333621]

106. Fischbach C, et al. Engineering tumors with 3D scaffolds. Nature Methods. 2007; 4:855–860. [PubMed: 17767164]

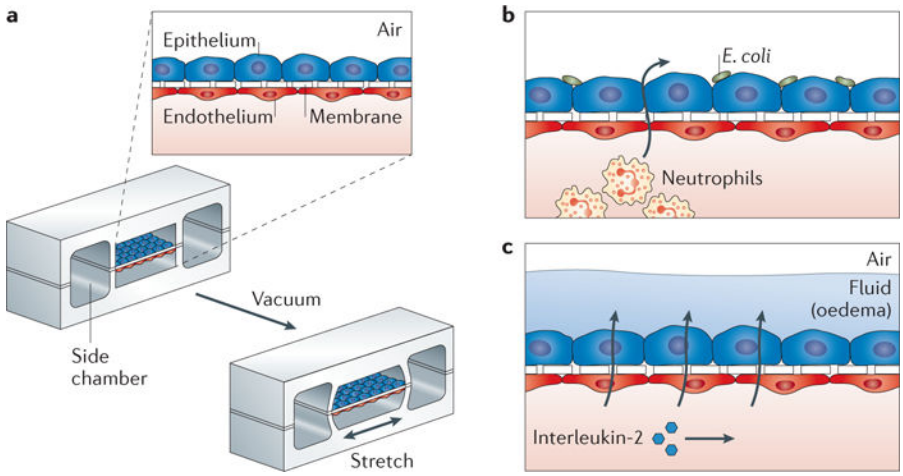

Figure 1. Lung-on-a-chip

a | A human breathing lung-on-a-chip was created by co-culturing human alveolar epithelial cells and pulmonary microvascular endothelial cells on opposite sides of a stretchable porous membrane to replicate the alveolar–capillary boundary of the breathing human lung. A vacuum was applied to mimic the tissue stretch that occurs during normal breathing. **b** | This system was used to reconstitute integrated organ-level functions such as inflammatory responses to intra-alveolar pathogenic bacteria such as *Escherichia coli* that are mediated by endothelial recruitment of circulating neutrophils, transmigration through the alveolar–capillary interface and subsequent bacterial phagocytosis. **c** | The lung-on-a-chip was used to model human lung diseases such as pulmonary oedema. Administration of interleukin-2 into the microvascular channel resulted in fluid leakage into the alveolar compartment, recapitulating the pulmonary oedema induced by acute toxicity of interleukin-2 that is observed in patients with cancer.

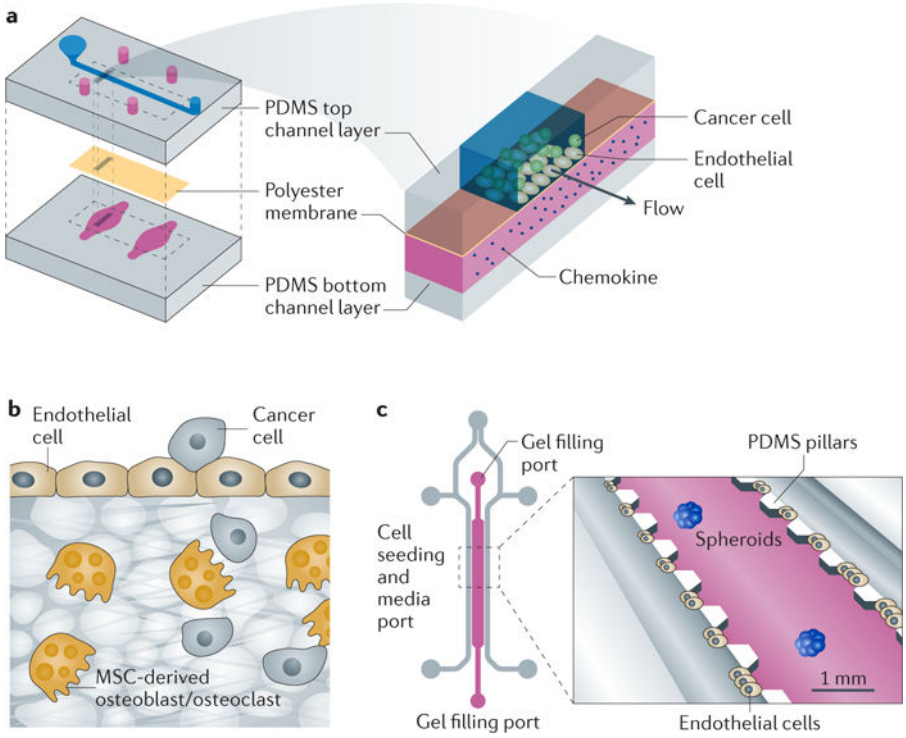

Figure 2. Organ-on-a-chip models for cancer research

a | A microvascular endothelium-on-a-chip created in a compartmentalized microfluidic device enabled basal stimulation and activation of endothelial cells grown on a porous membrane using chemokines to study the attachment of circulating breast tumour cells involved in cancer metastasis. The effect of chemokines, such as tumour necrosis factor, was investigated by adding these agents to the bottom channel. More cancer cells attached to the endothelium that was pre-treated with tumour necrosis factor than to untreated endothelium. **b** | The metastasis of breast cancer cells to bone was studied using human umbilical vein endothelial cells grown in a microfluidic channel adjacent to a 3D collagen gel containing bone cells differentiated from human bone marrow-derived mesenchymal stem cells (MSCs). Migration of the cancer cells into the bone was observed. **c** | To study epithelial–mesenchymal transition in cancer, lung cancer spheroids were embedded in micropatterned 3D matrices immediately contiguous to a microchannel lined with endothelial cells. Analysis of epithelial–mesenchymal transition is conducted using microfluorometry to detect dispersion of the cancer spheroids. PDMS, poly(dimethylsiloxane).

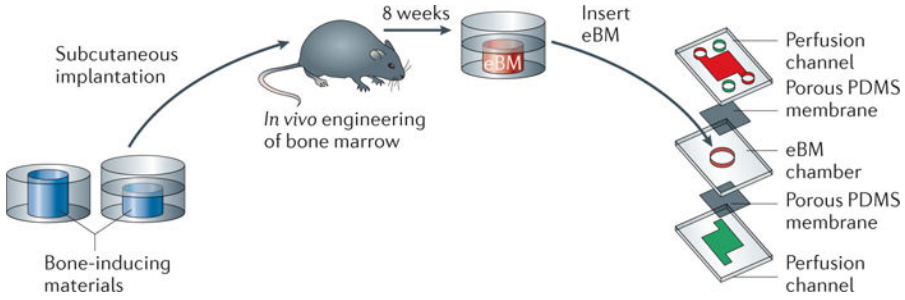

Figure 3. *In vivo* engineering of bone marrow

A bone marrow-on-a-chip model leveraged *in vivo* tissue engineering approaches to generate fully functional engineered bone marrow (eBM) for incorporation and perfusion culture in a microfluidic device. The eBM had a physiological structure and was used in an organ-on-a-chip system to study depletion and pharmacological protection of haematopoietic stem and progenitor cell populations exposed to γ-radiation. The red and green channels are medium perfusion channels used to maintain the eBM in the central chamber. PDMS, poly(dimethylsiloxane).

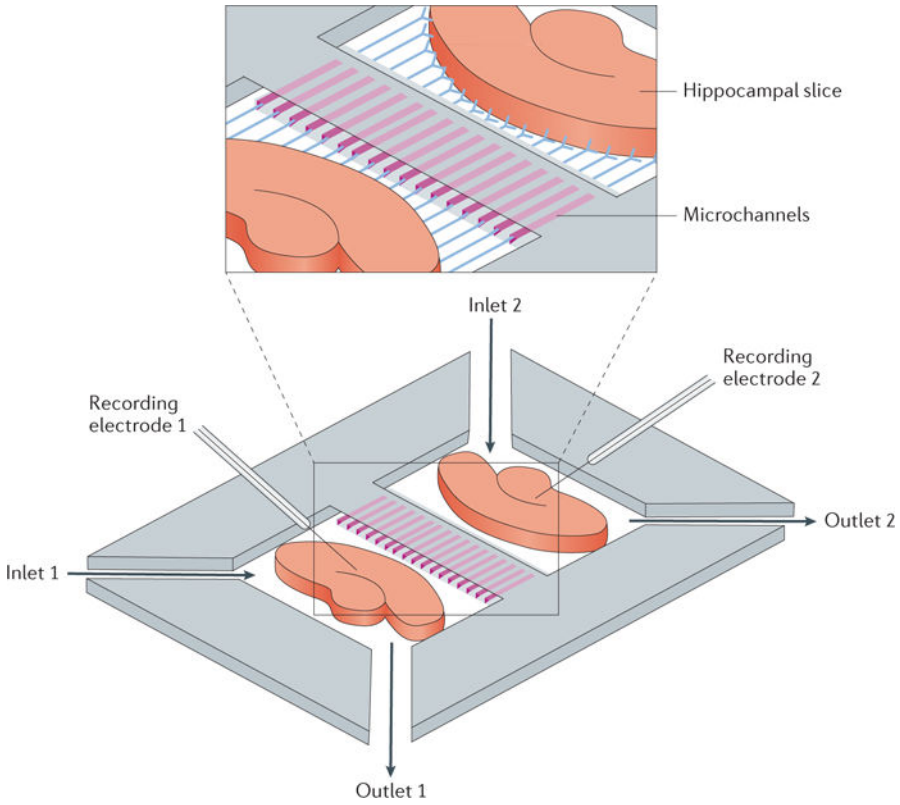

Figure 4. Brain tissue-on-a-chip

Higher-order functionality of the nervous system can be studied in a microdevice that enables culture of two brain tissue slices in separate media formulations. The two tissue slices are allowed to communicate by synaptic connections formed through microchannels between the culture chambers. Electrodes incorporated into this system enable measurement of synchronous electrophysiological activity between the two brain slices. This design allows for selective pharmacological treatment of only one tissue slice and measurement of its effects across the synaptic connections.

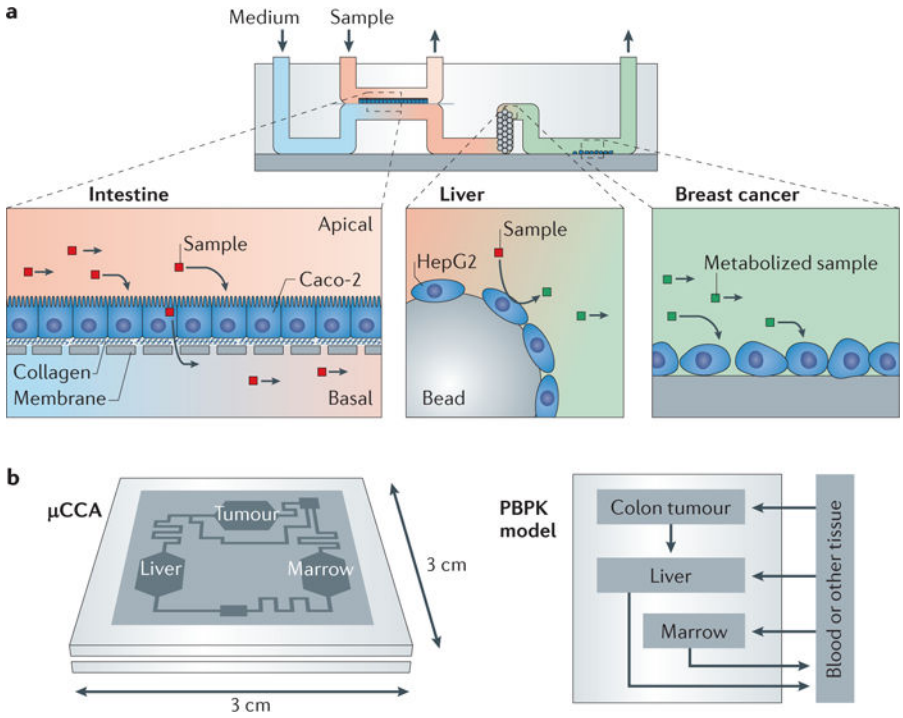

Figure 5. Body-on-chip systems

A | A microdevice containing interconnected cell culture microchambers was used to develop a multi-organ model that integrated microfluidic culture of intestinal epithelial cells, hepatocytes and breast cancer cells to simulate absorption, metabolism and activity of anticancer drugs. **b** | A micro cell culture analogue (μCCA) was created by linking together three interconnected microfluidic cell chambers representing a colon tumour, the bone marrow and liver with proportional physiological scaling to develop a more realistic physiological model of drug metabolism and anticancer activity. Culture media recirculates through the three inline chambers and an external reservoir, mimicking blood circulation and its residence times in the modelled organs. PBPK, physiologically based pharmacokinetic.

Table 1

Assays in organs-on-chips

Biological process/feature	Assay	Refs
Cell growth and death		
Proliferation	5-ethynyl-2′-deoxyuridine (EdU) labelling	56
	Ki-67 immunostaining	33
	Cell counting	44
Cell cycle	Flow cytometry	42,44
Viability	Alamar blue staining	39,55,60–62, 64,65,68,76
	Live/dead	55,64,65,68
	CellTiter-Blue * staining	60–62
	CellTracker Green * and CellTrace Blue AM * staining	39
Apoptosis	Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL)	38,43
	Hoechst 33342 staining	43
Cell and tissue morphology		
Myocyte elongation and alignment	Phase contrast microscopy	47,48
	Microfluorimetry	36,45–48
Epithelial polarity	Microfluorimetry	55,56
Collagen fibre alignment	Birefringence imaging	48,64
Intestinal villus crypt structure	Scanning electron microscopy	56
	Microfluorimetry	56
Tubulogenesis	Phase contrast microscopy and microfluorimetry	73
Cardiac remodelling	Reverse transcription PCR	47
	Whole-transcript microarray	47
Cell differentiation and maturation		
Epithelial–mesenchymal transition	Microfluorimetry	37
Protein localization	Microfluorimetry	56
Cardiomyocyte maturation	Real-time quantitative PCR	48
Haematopoiesis	Flow cytometry	40
Cell motility and contractility		
2D migration	Microfluorimetry	34
3D invasion and migration	Phase contrast microscopy	68,71
Cell aggregation	Microfluorimetry	34,68
Spheroid dispersion	Microfluorimetry	37
Extravasation	Microfluorimetry	13,33
Metastasis	Microfluorimetry	33
Cardiac contractility	Video microscopy	36,45–47
Barrier function		
Permeability	Transepithelial electrical resistance	15,55–57,62,81

Biological process/feature	Assay	Refs
	Reaction product fluorescence	56
	Fluorescence	62
	Microfluorimetry	56,62,81
	Phase contrast microscopy	15
	Mass spectroscopy	57
Intercellular junctions	Microfluorimetry	13,15,55,71,81
Oxygen transport	In-line fluorescent sensing	15
Nanoparticle translocation or uptake	Microfluorimetry	13,81
Intracellular drug accumulation	Microfluorimetry	39
Metabolism		
Enzyme activity and drug metabolism	Reaction product fluorescence	42–44,53–56
	Reaction product luminescence	56
	Microfluorimetry	56
	Mass spectroscopy	43,44,53,54
	NMR spectroscopy	42
Gene expression	Real-time quantitative PCR	44,53
Glucose synthesis	Colourimetry	42
Mitochondrial dysfunction	JC-1 mitochondrial membrane potential indicator dye	42
Secretion		
Albumin synthesis	ELISA	42
Mucus production	Alcian blue staining	56
Electrophysiology		
Myocyte activity	Microelectrode arrays	46,48
	Microfluorimetry	46,48
Neuron activity	Microelectrode arrays	41
Oxidative stress		
Reactive oxygen species production	Microfluorimetry	13,15,35

* Proprietary name of assay or dye.

Table 2

Three-dimensional cell culture matrices and scaffolds for organs-on-chips

Matrix or scaffold	Cells	Application	Refs
Collagen	Lung cancer cells	Epithelial–mesenchymal transition	37
	Breast cancer cells	Invasion	68
	Breast cancer cells, bone-differentiated mesenchymal stem cells	Invasion, metastasis	33
	Vascular endothelial cells, endothelial pericytes	Tubulogenesis	73
	Cardiomyocytes	Cardiac contraction	48
Matrigel	Hepatocytes, colon tumour cells	Drug metabolism, anticancer activity	64,65
	Hepatocytes, embryonic fibroblasts	Aggregate formation	68
Fibrin	Vascular endothelial cells, mesenchymal stem cells	Vasculogenesis	104,105
Alginate	Myeloblasts	Drug metabolism, anticancer activity	64,65
Poly(lactide-co-glycolide)	Oral squamous cell carcinoma	Tumour angiogenesis, anticancer activity	106
Foamed polylactic acid	Hepatocytes, brain cancer cells	Anticancer activity	76
Poly(ethylene) glycol	Hepatocytes	Drug metabolism	43