Organs-on-chips: into the next decade

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Abstract | Organs-on-chips (OoCs), also known as microphysiological systems or 'tissue chips' (the terms are synonymous), have attracted substantial interest in recent years owing to their potential to be informative at multiple stages of the drug discovery and development process. These innovative devices could provide insights into normal human organ function and disease pathophysiology, as well as more accurately predict the safety and efficacy of investigational drugs in humans. Therefore, they are likely to become useful additions to traditional preclinical cell culture methods and in vivo animal studies in the near term, and in some cases replacements for them in the longer term. In the past decade, the OoC field has seen dramatic advances in the sophistication of biology and engineering, in the demonstration of physiological relevance and in the range of applications. These advances have also revealed new challenges and opportunities, and expertise from multiple biomedical and engineering fields will be needed to fully realize the promise of OoCs for fundamental and translational applications. This Review provides a snapshot of this fast-evolving technology, discusses current applications and caveats for their implementation, and offers suggestions for directions in the next decade.

Drug development is slow and costly, driven mainly by high attrition rates in clinical trials[1]. Although remarkable increases in our understanding of the molecular underpinnings of human diseases and our ability to model in vivo cell, tissue and organ-level biology have been made over the past three decades, the number of US Food and Drug Administration (FDA)-approved drugs per billion US dollars spent on research and development has actually decreased monotonically since 1950 (REF.[2]). Drug development needs new approaches, paradigms and tools to reverse these trends and thus deliver on the promise of science for patients[2].

Although animal models have contributed enormously both to our understanding of physiology and disease and to the development of new medicines, researchers have long been aware of the frequent discordance between animal and human studies and therefore the need for modelling and testing platforms that would be more predictive of human responses[3,4]. Indeed, drug candidates may be terminated for lack of efficacy in animals, or discovery of hazards or toxicity in animals that might not be relevant to humans. Despite significant developments in computational and in vitro biology and toxicology in the past two decades, currently more than 80% of investigational drugs fail in clinical testing, with 60% of those failures due to lack of efficacy and another 30% due to toxicity[5].

To address some of these issues and offer alternative tools for preclinical stages, early 'cell culture analogues'[6,7] were explicitly designed to culture mammalian cells in linked chambers perfused with a recirculating tissue medium, or 'blood surrogate'. Following on from these models came a 'heart–lung micromachine', integrating a lung cell culture model with a cardiac device to assess the effects of drugs and therapeutics delivered to the human lung by aerosol on cardiac function and toxicity in vitro. This first 'lung-on-a-chip' research was published in 2010 (REF.[8]) and set the stage for organs-on-chips (OoCs; synonymously known as 'tissue chips' or microphysiological systems) — microdevices engineered to contain (human) cells and tissues and to model or mimic organ structures, functions and reactions to biological conditions, stressors or compounds.

The dramatic expansion of the OoC field in the past decade has been made possible by the convergence of multiple previously disparate technologies, including induced pluripotent stem cells (iPS cells) and mixed cell culture capabilities, genome editing, 3D printing, sophisticated cell sensors, microfluidics and microfabrication engineering, which led to the demonstration that dynamic culture conditions significantly influence the physiological maturation and function of in vitro systems. Tissue chips offer promise in, for example, modelling multiple organs and tissues from individual donors of both healthy and diseased disposition, and investigating the responses of these tissues to environmental perturbations and therapeutics with known or unknown mechanisms of action. Worldwide investment from scientific funding bodies (BOX 1) has enabled the development of a multitude of 3D tissue models, from

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Box 1 | **Collaborative tissue chip development efforts**

In 2010, the US Food and Drug Administration (FDA) and the US National Institutes of Health (NIH) created a Joint Leadership Council to help speed up the translation of biomedical discoveries at the laboratory bench to commercial availability of new therapeutics. Under this mandate, the Advancing Regulatory Science programme was initiated, with awards issued to address distinct, high-priority areas of regulatory science. On the basis of the promise from these funded projects, from which the seminal lung-on-a-chip work was published[8], the NIH and FDA partnered with the Defense Advanced Research Projects Agency (DARPA) to fund two 5-year programmes for the development of organs-on-chips (OoCs). The NIH programme, called 'Tissue Chips for Drug Screening' (see Related links), awarded funding to develop 3D microsystems to represent multiple tissue types and also concurrently funded a programme to explore the use of stem cells and progenitor cells for differentiation into the multiple cell types that would be needed to populate the microsystems. DARPA's microphysiological systems programme (see Related links) focused on developing a reconfigurable platform of at least 10 human organs or tissues in an integrated system that could mimic and replicate biological crosstalk between tissues. While both initial programmes ended in 2017, the NIH continues to offer funding for further development of OoCs in an expanding array of programmes, including for disease modelling, inclusion of immune factors, modelling of Alzheimer disease, use in the context of clinical trials and as part of the NIH Helping to End Addiction Long-term Initiative (HEAL Initiative; see Related links) to address the US opioid epidemic.

The FDA has offered advice and guidance from a regulatory standpoint for the past decade, and recently signed memorandums of understanding with a number of commercial tissue chip companies to onboard the technology to FDA laboratories. Additionally, the IQ Consortium (see Related links), a non-profit organization consisting of pharmaceutical and biotechnology company representatives, partnered with US government funding agencies in 2016 to add end-user stakeholder perspectives to the field. The IQ Consortium recently published a series of articles on the characterization and use of OoC systems in safety and toxicity profiling applications[56,160] and for modelling skin[161], lung[162], the gastrointestinal tract[163], kidney[164] and liver[165].

In Europe, the Institute for human Organ and Disease Model Technologies (hDMT; see Related links), headquartered in the Netherlands, leads the way on integrating state-of-the-art human stem cell technologies with biotechnical fields to support the development and validation of human organs and disease models on chip. The hDMT consortium helped coordinate one of the European Union's Horizon 2020 research and innovation programmes termed 'Organ-on-Chip Development' (ORCHID; see Related links), and in late 2018 launched the new European Organ-on-Chip Society (EUROoCS; see Related links), which will encourage development and coordination of tissue chip research in Europe. Other countries are following the hDMT example and are establishing similar organ-on-chip networks in Israel, the UK, the Scandinavian countries and Switzerland.

One key tenet of collaborative partnerships for tissue chip development has been the involvement of different stakeholders to help advance each of their missions. For example, partnership of tissue chip developers with the CiPA initiative (see Related links) helps provide tools to fulfil CiPA's mission of engineering assays for assessment of the proarrhythmic potential of new drugs with increased specificity compared with current assays, while demonstrating the utility of tissue chips for toxicity screening.

A collaboration between the NIH and the Center for Advancement of Science in Space (CASIS; see Related links) allows researchers to use the microgravity environment on the International Space Station (ISS) to conduct biomedical research. The programme, which partners with the ISS National Laboratory, is using microgravity as a tool to investigate Earth-based disease pathologies such as the formation of kidney stones that would otherwise be difficult or take too long to model on Earth. Moreover, researchers and space payload developers work collaboratively to adapt OoC platforms and make them robust enough for rocket launch, spaceflight, integration into ISS facilities and splash down. This is leading to advances in the technical engineering of robust platforms capable of higher throughput (more than 24 replicates running concurrently) with a much smaller footprint. The systems are turnkey enough to be 'astronaut-proof', meaning that non-scientist workers (in this case astronauts, most of whom are not trained in laboratory techniques) can perform the necessary interventions — both in space and in the future on Earth in a variety of applications[166].

relatively simple single cell-type organoids to complex multicell-type, multi-organ microfluidically integrated systems (TABLE 1). Consortia, committees and workshops have emerged in Europe, the USA and Asia to discuss state-of-the-science aspects of OoCs (BOX 1).

In this Review, we cover how OoCs have evolved over the past decade into a potentially transformational translational science paradigm. OoCs could impact drug discovery and development by offering novel tools for disease modelling and understanding, as well as providing alternative — and potentially more predictive — methods for assessment of the toxicity and efficacy of promising new compounds and therapeutics. There are clear opportunities for this technology to provide more rapid, cost-effective and accurate information on human diseases and drugs being developed to treat them, providing insights for academic, biopharmaceutical and regulatory scientists that were previously not possible. We will explain how OoCs can model healthy and diseased phenotypes and discuss the promise of linked platforms for the creation of 'body-on-chip' systems. Importantly, we will cover the limitations of OoCs and discuss how defining the context of use of OoC platforms is critical for their continued development. Current considerations and challenges will be detailed, and our predictions for the ongoing era of tissue chip research will be presented.

Key features of organs-on-chips

OoCs are bioengineered microdevices that recapitulate key functional aspects of organs and tissues. While there is wide diversity in the specific designs of each platform, OoCs range from devices the size of a USB thumb drive to larger systems that reflect multiple linked organs within the footprint of a standard 96-well laboratory plate. All OoC platforms have three critical and defining characteristics: the 3D nature and arrangements of the tissues on the platforms; the presence and integration of multiple cell types to reflect a more physiological balance of cells (such as parenchymal, stromal, vascular and immune cells); and the presence of biomechanical forces relevant to the tissue being modelled (such as stretch forces for lung tissues or haemodynamic shear forces for vascular tissues). One way that biomechanical forces can be introduced to model fluid flow across the tissues is to include microfluidic channels in the systems to deliver and remove cell culture media and to remove associated cell metabolites and detritus. Organoids — another type of multicellular 3D tissue model replicating some aspects of in vivo organ structure and function — are not classified as OoCs owing to their production through stochastic self-organization (rather than specific cell seeding and growth protocols) and lack of cytoarchitectural structure (rather than provision of scaffolding or specially shaped culture chambers)[9].

TABLE 1 highlights some specifics of how OoCs differ from 2D cell cultures. Each platform design, from 2D plates to complex 3D engineered systems, has advantages and disadvantages. Therefore, the selection of a particular platform will depend on the context of its use, such as the characteristics of the assays and their readouts. One key advantage of OoC platforms is the ability to control cellular and specific tissue architecture to emulate chemical gradients and biomechanical forces. This allows precise control over the biochemical and cellular milieu to model in vivo-like environments and responses. Other advantages include the ability to vascularize or perfuse tissues, either with inclusion of self-assembling endothelial cells that form perfusable lumens or by use of microfluidic channels that act as engineered vasculature, bringing nutrients and fluidic flow to cells within culture chambers. Also, the ability to incorporate real-time tissue function sensors such as microelectrodes or optical microscopy markers (for example, fluorescent biomarkers) allows cell health and activity to be monitored. FIGURE 1 illustrates some of the diversity of OoC systems and shows how they can provide a wide range of data outcomes that can be used during drug development.

Common considerations and challenges

Before OoC platforms are implemented, careful consideration of a large number of variables and challenges is needed to create and validate systems that reflect the context of use and desired outcomes. Although not mutually exclusive, these challenges can be categorized as either biological or technical.

Biological considerations and challenges

Defining context of use. When creating OoC systems, bioengineers are essentially reverse-engineering human cellular systems; that is, taking apart and analysing the components of the biological system, identifying the key aspects and components needed for function, and using these findings to reconstitute the functional system[10]. Reverse-engineering human tissues and physiological systems is complicated owing to an often-incomplete understanding of the composition and interplay of any given tissue and system. Therefore, rather than attempt to comprehensively model a complex system, it may be more useful to engineer simple tissues that can still give relevant and useful answers for the specific field of study. For example, it may be more beneficial to use discrete vascularized brain organoids[11–13] when one is modelling glioblastoma, psychiatric disorders or developmental neurotoxicity than to create a complex multi-organ system with cardiovascular, lymphatic and glymphatic components. However, a multi-organ system could provide novel pathological insights into disease mechanisms for disorders, or toxic effects that require interactions of more than one organ.

Currently, OoCs can model certain aspects of a tissue but no single system completely recapitulates a fully

Table 1 | **Key features of 2D and 3D engineered tissues**

Parameter	Conventional 2D systems	3D systems: Organoid	3D systems: Organ-on-chip
Production characteristics	Grown on rigid flat surfaces, often as a cellularly homogeneous monolayer	Embedded in hydrogels/suspended in ‘hanging drops’, and left to self-organize into multiple cell types	Multiple relevant cell types seeded into engineered chambers with perfusion and/or biomechanical forces included
Production complexity and speed	Generally straightforward and fast (minutes to days)	Generally straightforward, but slower (days to weeks) depending on cell sources	Variable complexity (depends on platform design), slower (days to weeks) depending on cell sources and required tissue maturation metrics
Level of control over cell architecture	High	Very low	High
Maturation of iPS cell-derived cells allowed by platform[a]	Immature	Improved but still highly immature	Platform designs can improve and encourage cell maturity[153]
Resulting cell morphology	Unnatural, with limited ECM composition and contact with cells	Size and shape similar to in vivo case, allows relevant ECM interaction during cell proliferation	Size and shape similar to in vivo case, allows relevant ECM interaction throughout cell lifetime
Diffusion of signal factors and nutrients	Short distances possible	Ineffective transport to interior can cause cell death or immaturity	Allows precisely controlled temporal and spatial gradients
Vascularization or perfusion?	Not possible, generally perfusion via medium change	Depends on cell types but likely creates non-functional vessels; externally perfused; can include fluid flow across tissue surfaces	Yes — by microfluidic channels or design which can include/create endothelialized vessels
High-throughput feasibility?	Yes	Possibly, depending on tissue[154,155]	Depends on platform design; generally low to medium throughput
On-platform assay and analysis difficulty	Low difficulty, easy access to cells and readouts	Tissue function analyses possible; cell separation not possible	Real-time tissue/organ function analyses possible
Variability and in vivo relevance of resulting tissues in manufactured platform	Low variability and relevance — simple, homogeneous cultures	Can show high variability and low relevance as there is little control over resulting cell subtypes and location	Can show low variability and high relevance — allows high levels of control over cell type and placement

ECM, extracellular matrix; iPS cell, induced pluripotent stem cell. [a]Immaturity of iPSC-derived cells is still a general issue.

functional and integrated human tissue, let alone an organ. Rather, systems are designed to model key aspects of a tissue — or its most characteristic features — to mimic the morphological and functional phenotype of interest, where the phenotype being evaluated depends on the question being asked. Despite the emerging diversity of OoC platforms (see[14] for a recent review), identifying the base platform choice that can provide

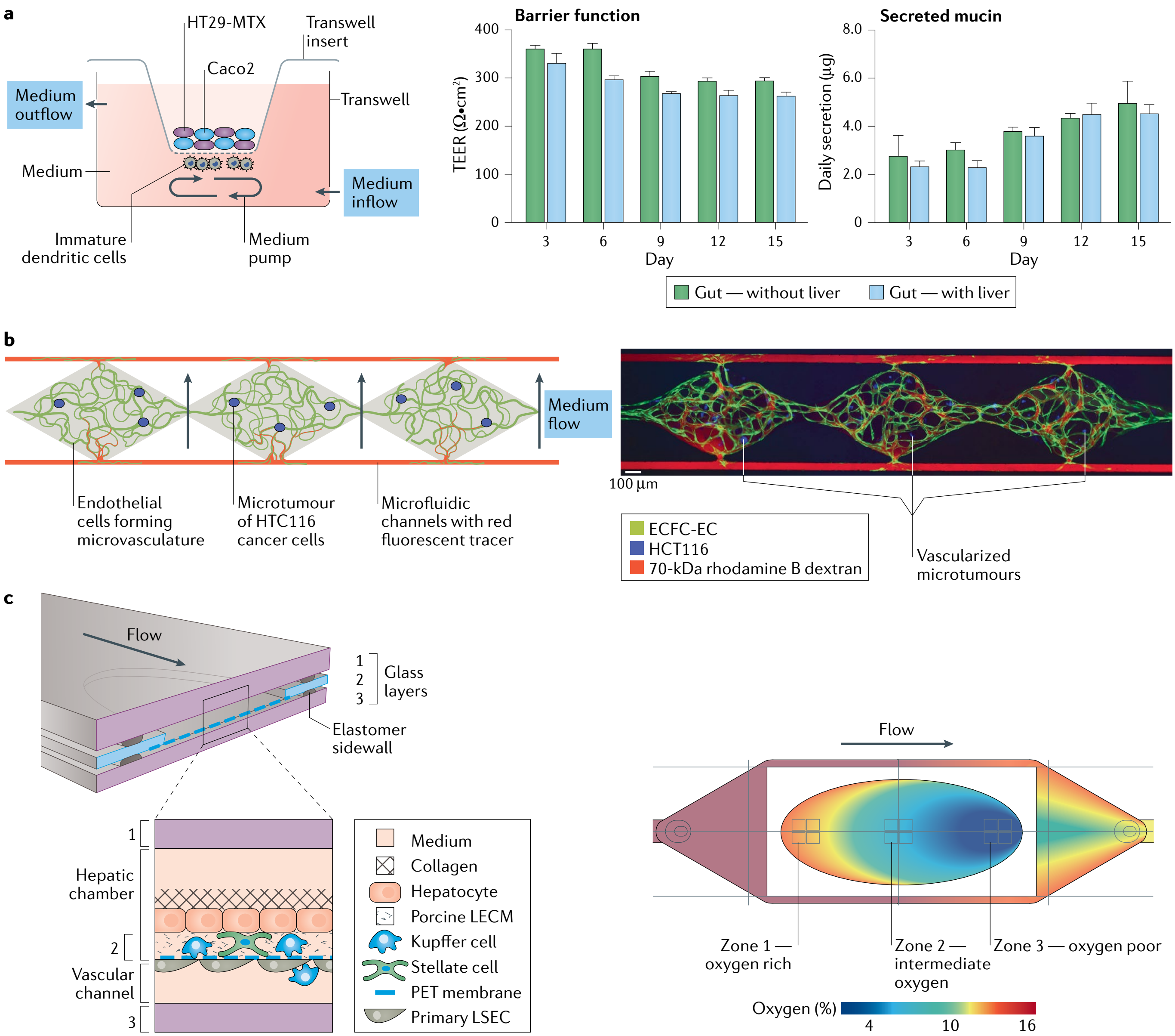

Fig. 1 | **Examples of features and platform designs for organs on chips.** Diverse platform design and key design features for organs on chips allow a broad range of data readouts that can be used for computational modelling as part of the drug discovery process. A broad diversity of tissue platforms highlights key common features — the three dimensions for tissue culture, inclusion of multiple cell types and modelling of biomechanical forces that recreate the in vivo environment. **a** | Transwell systems allow barrier modelling and fluid flow across a permeable membrane for medium exchange and cell–cell interaction. In this example, Caco2 and mucus-secreting HT29-MTX intestinal cells create the gut apical side, with immature dendritic cells seeded on the basal side and left to mature, creating a barrier model of the gut. On the right, barrier function of transwells can be measured by transepithelial electrical resistance (TEER) or secretion of, for example, mucin from cells in both single and linked organs-on-chips. **b** | Platforms with diamond-shaped cell chambers (2 mm wide and 1 mm high) allow seeding with human endothelial colony-forming cell-derived endothelial cells (ECFC-EC; in green), which self-organize into perfusable microvasculature, with cell medium supplied via microfluidic channels flowing from bottom to top. Seeding with colorectal cancer cells (HCT116 cells, in blue) forms vascularized microtumours that can be used to screen chemotherapeutic agents for safety and efficacy. Histology allows clear localization and visualization of cell interactions, such as the vascularization of microtumours and the perfusion of medium through the system (rhodamine B dextran, in red). **c** | A vascularized liver acinus model (left) consisting of cells in collagen sandwiched between three glass layers allows 3D layering of multiple liver cell types representing the liver acinus. Oxygen zonation can be computationally modelled by calculating the rate of medium flow in the microfluidic channels, creating three distinct zones (oxygen rich, intermediate oxygen and oxygen poor) on the platform, which recreate the liver sinusoid and establish a metabolic gradient similar to that seen in vivo (right). LECM, liver extracellular matrix; LSEC, liver sinusoidal endothelial cell; PET, polyethylene terephthalate. Part **a** adapted from REF.[140], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **b** adapted with permission from REF.[29], Royal Society of Chemistry. Part **c** adapted with permission from REF.[62], Royal Society of Chemistry.

Box 2 | **Cell sourcing for 3D tissue engineering**

The common aphorism of "all models are wrong but some are useful" is apt when considering cell sourcing for microphysiological systems (or any bioengineered tissue models). No cell source is perfect (many have serious caveats), but even the most problematic cell source can provide useful information if used appropriately on the basis of the question being asked. Cells seeded in tissue chips come from three main sources: commercially available cell lines; primary cells from human donors; and induced pluripotent stem cell (iPS cell)-derived sources.

Commercially available cell lines

Cell lines should have extensive validation of purity and viability when received from reliable sources (such as the American Type Culture Collection) and are often proliferative as well as easy to culture and transfect. These cells have clear and reliable culture protocols, generally respond in stable and predictable ways and will likely contribute to high reproducibility. Commercially available cells can be excellent sources of hard-to-find cell types, or when primary and iPS cell sources are unavailable. However, these cell lines are approximations for the primary cell types found in vivo and should be periodically evaluated to see how far from the primary cell phenotype the new generations are straying.

Primary cells

The clear advantage of using cells from human donors is that the cells capture the phenotype (presumably genetically and functionally) of the mature adult state. Primary cells can model disease pathologies when sourced from donors with certain diseases and can accurately reflect clinical population variance in their phenotypes. However, because genetic and epigenetic differences arise during a donor's lifetime, variability between donors or batches can be hard to identify and track. For some primary tissues (for example, neural cells), access from donors may not even be possible. In many cases, primary cells are available because the tissue has been removed or biopsied for diagnostic purposes and can be displaying pathological phenotypes. Primary cells also require specialized culture and media to retain their phenotypes, which can be problematic in linked tissue chip systems, as a common medium could prove suboptimal for the different tissues.

Induced pluripotent stem cells

Stem cell-derived sources are a potential solution to cell sourcing difficulties for tissue chips because they are potentially infinitely renewable and can be from either healthy or diseased populations. These iPS cells provide huge potential for populating tissue chips because individuals could have platforms created that model their tissues and disease phenotypes. This also allows creation of isogenic cell lines for genetic disorders, in which the resulting iPS cells can be genetically engineered to either harbour the disease-specific mutation or not harbour it, allowing opportunities to study the genetic impact of a disorder with unparalleled specificity.

The drawbacks of iPS cell-derived tissues include the immature or fetal phenotype (for example, cardiomyocytes, kidney and liver) of the cells, which can limit their utility. The time and resources needed for creation and passaging of cell lines, and later differentiation, are long (9 months or more for some neural tissues) and expensive compared with the ease of purchase and use of commercially available cells. Also, cells may retain an 'epigenetic memory' of their donor tissues[167] depending on the number of passages, which can limit directed differentiation for specific tissues. Finally, adult stem cells grown as organoids (for later seeding in organs-on-chips) represent only the epithelial component of the tissue, not the stroma or vasculature, limiting their application.

Extracellular matrix

(ECM). Supporting network of macromolecules providing structural and biochemical support to surrounding cells. Promotes cell adhesion and cell–cell communication and produces biochemical cues for tissue growth and maintenance. The ECM is tissue specific and in animal tissues consists of fibrous elements (collagen and elastin), and links proteins (laminin and fibronectin) and other molecules.

answers to the research problems in question remains challenging for end users.

Cell sourcing. Regardless of system complexity, one universal issue faced by OoC developers and users is renewable cell sourcing (BOX 2). Choosing the appropriate cells for a system is partly based on the context of use of the platform but is also often based on the availability of a particular cell source from commercial entities or from primary donors, which each have advantages and disadvantages. Increasingly, iPS cells or adult stem cells sourced from mass production of tissue organoids are seen as the answer to the lack of available primary cells[15], and iPS cells have some compelling advantages. For example, iPS cells offer an almost unlimited source of cells, and generating isogenic cell lines from them means that all tissues in multi-OoC platforms could be from the same donor[16,17], thereby addressing a key source of variability. However, to date, the phenotype of many iPS cell-derived differentiated cells, such as cardiomyocytes, is immature, and protocols for differentiation and maturation are non-standardized and can be difficult to reproduce (BOX 2).

Cell scaffolds. In addition to understanding a tissue's composition, engineering a tissue requires understanding the functional interplay of cell types and the effect of the scaffold or extracellular matrix (ECM) on the function of the cellular architecture[18]. OoCs may use decellularized scaffolds or seed cells within natural or synthetic hydrogels to create an environment conducive to cell growth, but the ECM composition and 3D arrangement affect cell survival, morphology and polarity[19–21] and so must be carefully chosen and engineered to promote the formation of appropriate tissue characteristics. The choice of the ECM material must be considered. Hydrogels (networks of polymers that swell with water application) are a widely used material because of their biocompatibility, support for cell adhesion and similarities to many soft tissues and in vivo ECM, but may be difficult to engineer and lack standardized protocols for creation. The complexities of modelling even relatively simple tissues with few cell types can be exponentially magnified when vascularization, innate or adaptive immune responses, and the frequent and often large variability in tissue sources between donors/suppliers/batches are included. Recent advances in bioengineering allow new possibilities for incorporation of biosensors into systems via the ECM. For example, incorporation of fluorescent microgels containing peptides that are cleaved in the presence of specific enzymes[22] offers the opportunity to use ECM for real-time readouts of OoC assays.

Linking multiple platforms. Linking multiple OoCs into multi-organ systems is not trivial and requires consideration of aspects such as biological (allometric) scaling, maintenance of sterility when building or connecting tissue modules, use of a common medium, incorporation of bubble traps and control of varying flow rates[23,24]. Additionally, a number of organs and tissues are necessarily missing from even the most complex series of linked OoCs, necessitating the need to account for missing organs. For example, how can a linked platform model important diurnal or endocrine fluctuations — which affect cell and drug metabolism[25,26] — if tissues producing or responding to those cues are absent? One solution has been the creation of complex engineered 'microformulators' to formulate, deliver and remove culture medium at defined time intervals, simulating the function of missing organs[27]. However, this remains an ongoing challenge.

Universal medium. Each tissue requires an adequate supply of specific nutrients and growth factors relevant for that tissue, so for linked OoC tissue systems, a key challenge is providing this kind of universal cell culture medium or 'blood mimetic'. So far, approaches

Hydrogels
Highly absorbent and hydrophilic biocompatible 3D polymer networks used to contain cells or drugs for tissue engineering applications. Can consist of natural (collagen, gelatin and agarose) or synthetic components and respond to environmental conditions such as pH. May have both liquid and solid properties. Other uses include wound dressings and contact lenses.

Multi-electrode arrays
Arrays of tens to thousands of tightly spaced microelectrical sensors designed to record from single cells to networks of cells on submillisecond timescales. Can also be used to stimulate cells with precise spatial and temporal characteristics. Used in electrically excitable tissues such as cardiac, muscle and neural tissues.

to address this issue have included scaling mixtures of culture media and engineering endothelial barriers. For example, circulating a 50:50 mixture of liver-specific and kidney-specific media in a linked liver–kidney system recently enabled the nephrotoxic metabolites of aristolochic acid to be determined[28]. However, as the number of linked systems increases, the success of the scaling solution decreases, as every tissue ends up with a suboptimal culture medium, which will impact the function and therefore physiological relevance of the system. Approaches for linking systems may involve creating single-pass or recirculating systems of culture medium that can be replenished or modified over time[29,30] or engineering platforms that allow culture of tissues in individual modules but provide access to a circulating 'blood surrogate' medium by inclusion of synthetic or endothelial barriers between tissue modules and the circulating medium[31–33]. Some researchers have approached the universal medium problem by providing tissues with appropriate individual support through variation of the surface chemistry of the platform or scaffold on which cells are cultured (for example, by silanes) while circulating a general serum-free medium to introduce fluidic flow to the system[34,35].

Technical considerations and challenges

Platform design. The characteristics of the assays that are intended to be run on an OoC must be considered early in the design phase or when one is choosing a particular platform. Many chips incorporate microfluidic channels, which can supply tissues with the nutrients and factors needed for function and introduce important biomechanical forces, such as the shear forces experienced by cells adjacent to vasculature. However, microfluidic designs must carefully model the resulting forces on the tissues because channel diameters, corners and input/output ports can influence the flow rate and therefore tissue performance[36]. Ports for inflow and outflow must be designed to maintain the sterility needed for cell culture while still allowing culture medium changes. Also, 'bubble traps' may need to be incorporated, as a bubble in a microfluidic channel can completely block all flow[37].

Modelling biomechanical forces is appropriate in certain tissues; for example, stretch forces for lung alveolar tissues[38]. An elegant solution from an early lung-on-a-chip introduced vacuum channels running alongside a porous membrane onto which lung alveolar cells were seeded on one side and lung endothelial cells were seeded on the other. Rhythmic application of the vacuum caused stretching and relaxation of the cell-lined membrane and mimicked the biomechanical forces associated with breathing[8]. This design has been adapted for many other tissues, including gut[39], heart[40], blood–brain barrier[41] and kidney glomerulus[42], highlighting how a simple design concept can be useful for multiple applications.

The assays of interest for each platform will ultimately dictate platform design. For example, chips replicating cardiac function likely need to allow access by a microscope and be fabricated from optically clear materials to allow imaging of cardiac twitching[43,44]. Liver chips modelling oxygen zonation may make use of microfluidic flow rates to create differing zones of oxygen saturation[45]. Neural or muscular (cardiac or skeletal) platforms should incorporate multi-electrode arrays or more microscale assays such as patch clamping or voltage clamping to provide readouts of cell activity[40]. Inclusion of biosensors such as fluorophores can allow real-time readouts of cell function; for example, metabolism, activity or activation of certain molecular pathways[46]. A recent automated multitissue organ system integrated an impressive array of on-chip sensors, including electrochemically activated immunobiosensors attached to physical microelectrodes, minimicroscopes and optical pH, oxygen and temperature monitors[47]. This technical feat highlights the ongoing engineering advances that are enabling real-time non-invasive monitoring of OoC microenvironments.

Platform fabrication. Although hydrogels and other scaffolds can help structure the internal cellular architecture of an OoC, the fabrication materials for the chip itself must be carefully considered. Every material for platform fabrication has a surface chemistry that affects how cells, fluids and compounds bind to or are absorbed into the material. For example, polydimethylsiloxane (PDMS) is a silicon-based organic polymer that is widely used for platform fabrication because it is affordable and easy to work with via soft lithography methods, allowing fast prototyping and easy iterative design change, and it creates flexible, biocompatible, optically clear platforms that allow modelling of biomechanical forces and real-time tissue imaging. However, PDMS is gas permeable (which can be an advantage or otherwise) and has high absorbance for small hydrophobic molecules[48]. Therefore, PDMS is problematic for drug studies, as the PDMS-based platform itself can absorb a large amount of the drug, or the resulting factors released from the cells may be leached from the effluent. There is also a risk of cross-contamination of chambers or channels adjacent to each other. So, mitigatory approaches for PDMS OoCs include treatment or coating of the polymer-based surfaces of the device to prevent cell adhesion or drug loss[49–52]. Alternative materials for chip fabrication include glass, silicon and thermoplastics such as cyclic olefin copolymer and poly(methyl methacrylate), with the material choice often being a trade-off between the needs of the platform and the availability, affordability or fabrication feasibility of the materials.

Regardless of the fabrication material choice, all OoC platforms require careful characterization of adsorption/absorption profiles. Additionally, the biocompatibility of the materials to be used must be considered and profiled, as unexpected toxic effects could appear when one is repurposing materials for platform fabrication[53].

Organs-on-chips for toxicity assessment

Toxicity and unknown safety for exposure to human tissues are large sources of failures of potential drug candidates, and accounted for 40% of losses based on failure data from four large pharmaceutical companies[5]. Traditionally, key individual tissues that are targeted for toxicity assessments include liver, heart, kidney, vasculature and brain. Methods of assessing toxicity in

Table 2 | **Examples of single tissue OoCs for toxicological assessment**

Tissue/ organ	Platform characteristics	Challenge	Response	Ref.
SQL-SAL model	Human hepatocytes and stellate, immune and endothelial cells are layered in glass and PDMS microfluidic chip Fluorescent biosensors included Survival to 28 days	Troglitazone and nimesulide (hepatotoxic) Trovafloxacin plus LPS and levofloxacin plus LPS (immunomediated hepatotoxicity) Methotrexate (fibrotic injury) Caffeine (negative control)	Time- and dose-dependent LDH release, apoptosis, plus decreased albumin and urea secretion Increased LDH release and apoptosis with trovafloxacin plus LPS but not with levofloxacin plus LPS Increased levels of fibrotic markers No effect	Vernetti et al.[61]
Liver	Primary hepatocytes placed across porous membrane from LSECs, with or without Kupffer and stellate cells Rat, dog and human species comparisons possible	Bosentan (cholestatic) Acetaminophen (hepatotoxic) Methotrexate (fibrotic injury)	Species-specific albumin decrease; correlated to clinical response in humans; bile salt transport inhibition Glutathione and ATP depletion; formation of ROS; decreased albumin secretion Lipid accumulation (steatosis) and fibrosis	Jang et al.[63]
Cardiac	Self-organized iPS cell-derived cardiomyocytes in 3D microfluidic device	Isoproterenol (β-adrenergic agonist) E-4031 (hERG blocker) Verapamil (multi-ion channel blocker) Metoprolol (β-adrenergic antagonist)	Cardiac beat frequencies in line with clinical data including dose-dependent changes and arrhythmias concordant with human cardiotoxicology data	Mathur et al.[64]
Kidney	Primary human kidney proximal tubule epithelial cells seeded to form a lumen in microfluidic platform	Polymyxin B	Increased KIM1 and injury-associated microRNAs	Weber et al.[78]

ATP, adenosine triphosphate; hERG, human ether-a-go-go-related potassium channel; iPS cell, induced pluripotent stem cell; KIM1, kidney injury molecule 1; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; LSECs, liver sinusoidal endothelial cells; OoCs, organs-on-chips; PDMS, polydimethylsiloxane; ROS, reactive oxygen species; SQL-SAL, sequentially layered, self-assembly liver.

these organs often use high-throughput but simple cell culture assays, which cannot replicate a complex systemic response to a compound, or animals, which can model complex responses but may not provide an accurate prediction of effects in humans. Pharmacokinetic/pharmacodynamic modelling and physiologically based pharmacokinetic modelling can be used to predict the absorption, distribution, metabolism and excretion (ADME) of chemical substances in the body. However, these modelling methods rely on data from other model systems and detailed anatomical and physiological information where it is available. Animal studies are crucial for studying systemic and longer-term effects in full biological systems, but the similarities and differences in comparative physiology with regard to humans can be anywhere on the spectrum between directly translational to confounding or even completely unknown. Indeed, extreme and sometimes tragic examples of the difficulty in translating findings from animals to humans can be seen in high-profile phase I clinical trial failures, although these events are thankfully rare[54,55]. These failures were seen either during the 'first-in-human' phase[54] or during the dose escalation phase. The drawbacks of current toxicity profiling highlight the intricacies of the translational process from cell culture to animals and ultimately to humans, which can place clinical trial volunteers at high-risk however carefully planned and executed a trial is. Additionally, there is a growing need to predict the toxicity of novel modalities such as biologics, oligonucleotides and large molecules (molecular mass greater than ~900 Da) that are challenging or impossible to assess in standard animal models. OoCs may have advantages for these modality-specific assessments by allowing modelling of complex human responses in tightly controlled in vitro systems that may be linked to model organ crosstalk[56] and can be designed for specific contexts of use[57].

Pharmacokinetic/ pharmacodynamic modelling
Integration of pharmacokinetics (movement of drugs through the body) and pharmacodynamics (the body's biological response to drugs) into a mathematical model describing dose–concentration–response relationships. Can be used to predict effect and efficacy of drug dosing over time.

Physiologically based pharmacokinetic modelling
Mathematical modelling of body compartments (predefined organs or tissues) combined with known parameters of concentrations, quantities and transport between compartments used to predict absorption, distribution, metabolism and excretion of synthetic or natural chemical substances within the body.

Single-tissue OoCs offer an alternative way to approach toxicity assessments of potential compounds in various complex human 3D tissues[58]. In 2D liver cultures, hepatic cell line cultures poorly represent primary human hepatocytes[59], and the latter cells rapidly dedifferentiate over 24 hours[60], limiting their usefulness in evaluating either short or long exposure effects and systemic toxic effects. An example of how OoCs could address such issues is a recently developed 3D liver OoC system that can maintain healthy cell cultures for more than 28 days (TABLE 2) and mimic the in vivo environment of the liver (to include haemodynamic flow, oxygen zonation and inclusion of immune components)[61,62], which opens new pathways for ADME/toxicity studies. Oxygen zonation in this liver platform was achieved by controlling the flow rate of the medium through the platform to create zones of differing oxygen tension, and coupling computational modelling of this tension to direct temporal and spatial monitoring of oxygen-sensitive dyes in the system[45]. This highlights how use of biomechanical forces and direct experimental assays from real-time biosensor readouts can be combined to provide powerful tools for accurate replication of clinically relevant toxicity profiles. Separation of the sinusoid (vascular channel) and hepatic compartment by a porous membrane allows physiologically relevant addition of drugs, immune cells and other factors to the model[62]. Another recent study comparing a liver on a chip from rat, dog and human cell sources elegantly showed species-specific differences in hepatotoxicity, highlighting the importance of using human-specific cells for certain assays, while confirming the validity of the use of non-human models for others[63] (TABLE 2).

For the heart, which is another important target organ of toxicity, a number of heart-on-a-chip systems have been developed that model the complex matrices of cardiomyocytes, (cardiac) fibroblasts, endothelial cells and vasculature that interact in vivo in a highly ordered manner, which can be easily perturbed by drugs, drug–drug interactions or off-target side effects. Since in vitro screens are now an integral part of drug development to characterize cardiac safety liabilities, the current heart-on-a-chip systems are useful as they model human responses to injury (TABLE 2), and show appropriately aligned sarcomeres, rhythmically synchronized beating patterns and physiologically relevant resting membrane potentials[44,64–67]. Other structures in the heart, such as cardiac valves, have been bioengineered to assess the off-target cardiac side effects of dopamine/serotonin production/reuptake-influencing drugs, such as pergolide, which are used in clinical treatment for psychiatric disorders such as Parkinson disease[68]. However, a large problem with all cardiac OoC systems currently using iPS cell-derived tissues is the fetal phenotype of most resulting cardiomyocytes[69,70]. Despite this, recent advances using electrical and mechanical stimulation to 'train' the developing cells or cardiac 'organoid' growth in fatty acid-based culture medium and inclusion of other relevant cell types seems to encourage a significantly more mature phenotype[71–74], further expanding the potential use of OoCs in the cardiotoxicity field.

Other important tissues for toxicity profiling include those from the kidney, gut and lung. Developmental toxicity assays, including neurotoxicity assays, are also relevant for many exposure studies. OoC models of the kidney (nephron and proximal tubules) can be used to model readouts relevant for nephrotoxicity profiling such as filtration, reabsorption, transport of various molecules and action of protein transporters[75–78]. Indeed, a kidney-on-a-chip system was used to elucidate that polymyxin B nephrotoxicity may be caused by the cholesterol biosynthesis pathway, highlighting how OoCs could be used not only to test the safety of novel chemical molecules but also to shed light on toxicological pathways of FDA-approved molecules[78] (TABLE 2). Gut-on-chip systems can model certain aspects of the bioavailability and activity of drugs by the creation of in vitro intestinal epithelia and exposure of these tissues to relevant biomechanical forces, such as flow and peristalsis[79,80]. Inclusion of immune and microbiome factors becomes critical for true human relevance, both of which by themselves are huge areas of research, although there is progress being made in inclusion of these in both organoid systems[81] and microfluidic systems[82–85]. For example, the HuMiX model to recreate human–microbial crosstalk allows researchers to investigate the causal relationships between the gastrointestinal microbiota and certain human diseases, but could also be used in toxicology and pharmacokinetic studies[82]. Toxicity profiling of inhaled substances can benefit from lung-on-a-chip models that can recapitulate the air–liquid interface of the lung alveoli[8,86] and model effects such as exposure to bacteria, drug-induced pulmonary oedema and cigarette smoke[87]. Developmental neurotoxicity can be modelled in platforms containing 3D neural tissues. For example, in a study that used RNA-sequencing readouts from neural constructs exposed to 60 drugs of known toxicity, a predictive model based on linear support vector machines had more than 90% accuracy in predicting the toxicological impact of 'blinded unknown' compounds[13], highlighting the potential power of these types of 3D models for predictive toxicology. Other developmental toxicological vulnerabilities have been assessed with use of placenta-on-a-chip models that can recapitulate the ability of compounds to cross or affect the maternal–fetal barrier[88,89]. Readouts of vascular-related toxicity may be critical for therapeutics, and vascular networks on OoCs have been used to investigate vascular toxicity with chemotherapeutics[29,90] and risk factors for complications such as thrombosis from monoclonal antibody treatments[91].

Finally, linked multi-organ systems could expand OoC applications into organ interactions and systemic toxicity profiling, and these are discussed further later.

Disease modelling on a chip

In addition to being useful as tools for understanding toxicity in human tissues, OoCs also offer ways to model disease states in vitro, thereby allowing mechanistic investigation not only of disease pathologies but also of the efficacy and potential off-target effects of therapeutic interventions. The potential enhanced understanding of human disease physiology from modelling diseases on OoCs could help address the high attrition rates of promising compounds seen during both lead optimization and clinical development stages due to lack of efficacy[5,92].

Stem cells and tissue chips — powerful partners

While many OoCs have been developed to model disease phenotypes using primary or cell line sources, the increasing use of iPS cells, plus the novel option of using the mass production of organoid technology as a way to source adult stem cells in biomedical research, has also led to the increased development of an array of diseases-on-chips, including cardiac (atrial and ventricular) myopathies[72,93,94], asthma[95], vascular abnormalities[96] and polycystic kidney disorders[97] as well as neural disorders — including those mimicking aspects of neurodegenerative and psychiatric disorder phenotypes[98,99] — and rare paediatric diseases such as Hutchinson–Gilford progeria syndrome[100]. However, a limitation associated with the use of stem cell-derived cells in OoCs is the difficulty in producing an adequate number of mature, differentiated cells with the necessary purity for many tissues (for more details, see BOX 2).

Despite these current limitations, one early example showing the power of the use of iPS cells in OoCs, coupled with genome editing technologies, investigated the rare childhood paediatric cardiomyopathy Barth syndrome. Stem cell-derived cardiac tissues from patient donors were created and modelled on 'muscular thin films', which replicated the disordered sarcomeric organization and weak contraction properties seen in the disease[101]. With use of genome editing techniques to 'correct' the faulty *TAZ* gene in the iPS cell-derived cardiomyocytes, mitochondrial abnormalities underlying the disease were identified. These results highlight the potential use of OoCs as models for the critical stages of target validation where the creation of multiple tissue types from the same patient, and the generation of isogenic control tissues by genetic editing methods for any number of genetically based diseases, can enable detailed and specific mechanistic studies for these disorders[102].

'You-on-a-chip' for common and rare diseases

Disease modelling on OoCs could contribute to the development of precision medicine. OoCs modelling angiogenesis[103], tumour growth[104] and intravasation and extravasation[105,106] have all contributed to the development of vascularized and metastatic breast cancer models[107–110]. The treatment of patient-derived tumours on chips with chemotherapeutics enabled treatment comparison and optimization[108], which is a step towards using this technology for precision medicine. Tumour-on-a-chip platforms have also helped parse the mechanistic effects of different chemotherapeutic agents on the resulting 'microtumours'[90]. Other tumour-on-a-chip models include neural glioblastoma[111], renal cell carcinoma[112] and lung[113], pancreatic[114], colorectal[115], ovarian[116], prostate[117] and cervical[118] cancer.

While many of these models were created with cancer cell lines, an obvious and powerful opportunity arises when patient-derived primary or iPS cell derivatives are seeded onto OoC models, creating 'patient-on-a-chip' models. This could inform the stratification of the population of cancer patients into subpopulations that respond optimally to different chemotherapeutic regimens or cocktails, but could also lead to development of 'you-on-a-chip' for patients with rare cancers or cancers with unusual causes. Communities with rare diseases could benefit tremendously from the opportunity to recreate these diseases on chips (see[119] for a review). For example, patient-derived pancreatic ductal epithelial cells can be used to create a pancreas-on-a-chip to potentially understand the cystic fibrosis transmembrane conductance regulator protein and its role in insulin secretion[120]. If iPS cell protocols become available for pancreatic cell creation — a current challenge with promising progress in the field[121] — then modelling of an individual with cystic fibrosis on a chip will become possible, which could prove useful in understanding the high risk of diabetes and glucose imbalance in this population.

Synergistic engineering to combine 3D models

Both OoC and organoid 3D models have strengths and limitations (TABLE 1), but innovative ways to combine the technologies and introduce related technologies such as 3D bioprinting — so-called synergistic engineering[122] — adopts strengths from multiple 3D bioengineering fields to create reliable predictive tissue models with the opportunities for higher-throughput screening (see[123] for a comprehensive review). For example, both organoids (which self-organize into three dimensions) and bioprinted tissues (where cells are deposited in a specific manner) can be seeded or printed in multiwell plates with medium flow and inclusion of other biomechanical forces, creating platforms with multitissue components that are amenable to larger-scale commercial production. An example of these combined technologies includes vascularized organ 'buds' that can be perfused by a common medium[124] and bioprinting of endothelialized myocardium in a microfluidic perfusion bioreactor[125]. In the case of the latter, multiple bioengineering techniques were combined to create an innovative tool for predicting cardiovascular toxicity. First, endothelial cells were encapsulated into bioprinted microlattices to allow formation of an endothelial vascular bed, after which cardiomyocytes were introduced, forming a myocardial tissue with good alignment to the bioprinted vascular bed. Finally, inclusion of the tissue construct into a microfluidic bioreactor allowed continuous vascular perfusion and real-time monitoring of cardiac contraction phenotypes for up to 2 weeks.

As with all disease models, the demonstration that these 3D tissue models effectively mimic the behaviours of the disease, as well as the responses to therapeutic drugs in vivo is critical for their validation.

Creating a 'body on a chip'

Linkage of multi-organ tissue systems is of clear benefit to model complex organ–organ interactions and inform pharmacokinetic/pharmacodynamic and physiologically based pharmacokinetic modelling, ADME profiling, quantitative systems pharmacology (QSP) and other computational modelling. Over the past decade, many efforts have been undertaken to integrate multiple systems and overcome the challenges associated with this (see[126] for a review). Indeed, US governmental funding from the Defense Advanced Research Project Agency (DARPA) was specifically allocated to create and link 10 organ systems (see Related links) that were viable for 28 days into a single 'body on a chip' as part of broader efforts by the US National Institutes of Health (NIH), FDA and DARPA to fund the development of tissue chips to advance regulatory sciences (see Related links). From this funding, two recent publications showed how a 10-organ 'physiome on a chip' combined with QSP computational approaches could model the distribution of in vitro pharmacokinetics and endogenously produced molecules[127] and how a robotic 'interrogator' maintained the viability and organ-specific functions of eight vascularized, two-channel organ chips (intestine, liver, kidney, heart, lung, skin, blood–brain barrier and brain) for 3 weeks in culture[128].

The study of prodrugs[129], which are metabolized by the body from inactive compounds to active compounds, could benefit, as could the development of novel compounds that rely on (or cause) bioactivation[130].

Slow-release mechanisms (for example, slow-release painkillers and contraceptive injections or implants), or compounds produced by non-traditional methods such as synthetic biology or genetic engineering, could also be extensively assayed for unexpected side effects. Coupling these types of new molecular technologies with powerful computational modelling tools, including QSP[131], machine learning[13] and artificial intelligence[132], could offer novel and helpful insights for current toxicological assessment. For example, capecitabine and tegafur (anticancer prodrugs) have been shown to be effective in a multi-organ pneumatic pressure-driven platform[133], and recently Boos et al.[134] used a hanging-drop organoid system to test how products metabolized by human liver microtissues affect embryoid bodies. The prodrug cyclophosphamide (activated by cytochrome P450) was added to the system, and a 50% drop of embryoid differentiation was seen, demonstrating how powerful synergistically engineered microfluidic systems can be not only for prodrug investigation but also for investigation of embryotoxicity in this case.

Challenges with linking systems include how to scale the organs of interest (for example, allometrically, based on body size, or metabolically[24]), model fluid flow dynamically through the system and scale flow appropriately for each tissue[23], supply all tissues with adequate growth factors and culture medium support (for example, via a blood surrogate culture medium[7] or by separation of cultures by endothelial barriers[135]) and design and fabricate these complex systems. One approach to linking systems that avoids many challenges faced by physically linking organ cultures involves functional coupling, such as running media through physically separate systems sequentially to model multi-organ ADME. In the case of Vernetti et al.[136], this approach showed that organ-specific processing of the tested compounds was consistent with clinical data, and additionally found that a liver-bioactivated microbiota metabolite crosses the blood–brain barrier via a neurovascular unit OoC[137,138].

A number of systems physically linked via microfluidics and pneumatic or peristaltic pump mechanisms have been published (FIG. 2) and include systems that have revealed, for example, novel mechanisms of aristolochic acid nephrotoxicity[28], the metabolic coupling of endothelial and neuronal cells in the neurovascular unit[139] and inflammatory crosstalk between the gut and the liver[140]. For example, Chen et al.[140] examined an integrated gut–liver transwell OoC and showed that modulation of bile acid metabolism was seen in the linked system. Meanwhile, in an inflammatory state (modelling endotoxaemia by increasing circulating lipopolysaccharide levels), hepatic biotransformation and detoxification pathways showed changes, highlighting that even relatively simple OoC models can give valuable information on organ interactions.

Additionally, a number of multi-organ systems demonstrating utility in toxicology and disease modelling applications are appearing in the literature, including systems modelling homeostatic mechanisms[32,141], hepatic metabolism and off-target cardiotoxicity[34,142], and the female reproductive tract and menstrual cycle[143], which reproduced a 28-day hormonal cycle in a platform including ovarian tissue, fallopian tube, uterus and cervix, but also included a liver module for reproductive toxicology utility (FIG. 2a). Synergistically engineered multitissue organoid-based platforms linked by microfluidic channels are also joining the expanding cadre of multi-organ OoC tools[47,133,144,145]. Importantly, many of these systems incorporate a variety of real-time assays and biosensors for ongoing cell health and function readouts and can support extended cell culture (less than 28 days), allowing long-term and repeated testing of compounds for systemic toxicity evaluation[35,146]. Some of these linked systems are becoming more broadly available to researchers either through contract research organization (CRO)-based services or through purchase of off-the-shelf systems, although the latter are generally simpler organoid-based higher-throughput multiwell plate systems. Manufacturing the more complex OoC systems designed by engineering laboratories is still an obstacle to widespread implementation in biomedical laboratories.

Replication, validation and commercialization

As OoCs become increasingly commercially available, reproducibility of the technology at multiple sites is becoming critically important. Negotiating legal frameworks to facilitate sharing of proprietary information and technologies between organizations can be lengthy. Meanwhile, sometimes critical exchange of reagents and trained personnel can be costly, and unexpected obstacles can emerge from simple processes such as shipping cells and resources. Some questions that arise from these obstacles include the following: Should cells be shipped in differentiated or undifferentiated forms? Should platforms be seeded with cells, or should the recipient fabricate the systems from shared moulds instead? Can cells be shipped in OoC plates in a frozen state and simply thawed before use by end users? Thorough consideration of the most straightforward processes can become complex and expensive.

Robust, reproducible, reliable platforms

The US government has provided almost a decade of support for OoC development, and although the DARPA body-on-a-chip programme has now ended other federal agencies continue to support US-based OoC development, and agencies in Europe and elsewhere are also supporting OoCs (BOX 1). In particular, the US National Center for Advancing Translational Sciences (NCATS) has created two new programmes since 2016 that focus on creation of reproducible, reliable and automated systems that are accessible to the wider community. The Tissue Chip Testing Centers (see Related links) initiative began in 2016 to support two independent centres charged with onboarding developers' tissue chips, monitoring reproducibility of assays and outcomes, and investigating additional parameters that are of use to the community. The first publication addressing independent validation of a kidney proximal tubule model was recently published[147], and several more are forthcoming. To encourage the development of robust automated systems with smaller laboratory benchtop footprints, the NCATS Tissue Chips in Space programme also promises advances for technical development in the field (BOX 1). These programmes, plus commercial pressures, are pushing the move towards more turnkey OoCs to help reduce or remove the need for the specialized infrastructure and highly skilled personnel that are currently often required for OoC implementation.

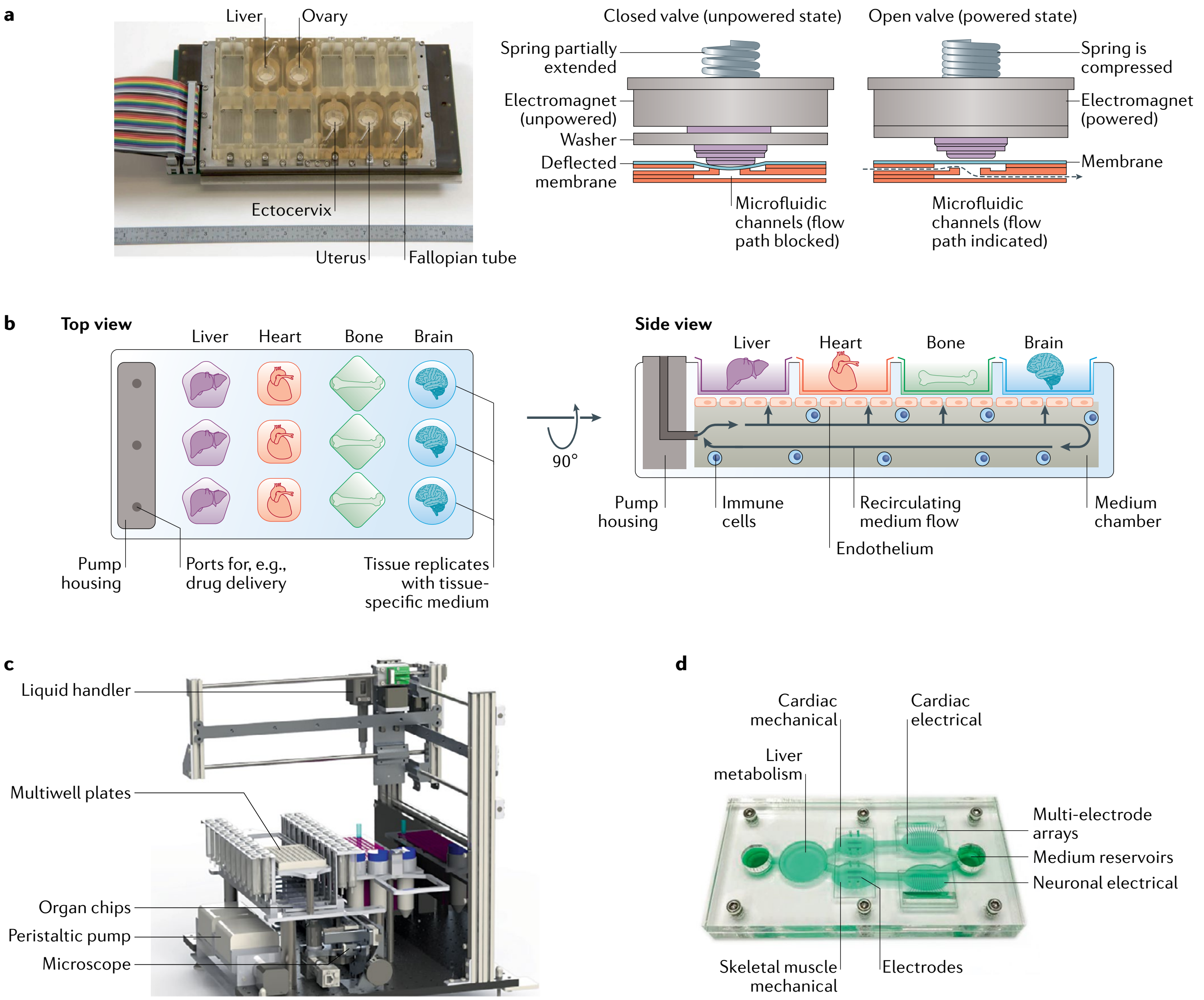

Fig. 2 | **Examples of linked multi-organ systems, which can help understand systemic or off-target drug effects and create 'body-on-a-chip' systems.** The modules and medium can be linked by pneumatic or electromagnetic pumps (part **a**), peristaltic flow (parts **b**,**c**), or medium circulated by hydrostatic flow driven by gravity (part **d**). **a** | This female reproductive system microphysiological system (left) contains five tissue modules (ovary, cervix, uterus, fallopian tube and liver) and models the hormonal profile of the female menstrual cycle and pregnancy, which can be useful for assessing female reproductive toxicity. The modules are linked by a complex series of internal valves and pumps under the tissue construct inserts and flow of tissue-specific media and hormones is driven by pneumatic pumps powered by electromagnets (right). **b** | A simplified schematic of a linked multi-organ system for investigating doxorubicin-induced toxicity in liver, heart, bone and various other tissues (for example, brain) is shown on the left. The platform consists of individual tissue constructs cultured in multiple modular 'inserts', set into a platform with the same footprint as a standard six-well laboratory plate. In this example, four tissue types can be replicated in triplicate on a single plate. A schematic of the side view of the platform is shown on the right. Underneath each tissue insert lies a permeable membrane lined with endothelial cells, perfused by a recirculating vascular medium driven by a peristaltic pump. The system allows optimal cell culture for each tissue type as well as inclusion of common circulating factors such as immune cells, hormones and exosomes. **c** | A robotic system with an inbuilt microscope, peristaltic pump and automatic fluid handling named the 'Interrogator' can house up to 10 organs-on-chips for pharmacokinetic/pharmacodynamic and physiologically based pharmacokinetic modelling. **d** | This commercially available multi-organ system from Hesperos Inc. cultures liver, cardiac and skeletal muscle and neurons on a microfluidic chip. Each tissue module is cultured on a plate modified by proprietary surface chemistries to help cells adhere to the surface and act as extracellular matrix, and medium reservoirs contain a serum-free common medium that is gravity fed by placing the chip on a laboratory rocker. Cardiac, skeletal and neuronal modules contain multi-electrode arrays to stimulate and record activity in tissue subtypes. Part **a** is adapted from REF.[143], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **b** (right) adapted with permission from REF.[156], Elsevier. Part **c** adapted from REF.[128], Springer Nature Limited. Part **d** adapted with permission from REF.[157], AAAS.

Commercial considerations and hurdles

Increasing throughput. Most complex non-organoid tissue chips are currently of very low throughput, where only dozens of replicates (at most) can be performed at any one time. Consequently, during the early stages of drug discovery, at which time many thousands of potential hits can be identified in a short time frame through standard high-throughput screening assays, the use of such chips is likely to be considered cost- and time-prohibitive for pharmaceutical companies at present. Technological advances to create more automated, miniaturized OoC systems that can become turnkey technologies for facile use will be crucial to increasing throughput and the number of replicates per platform.

Scaling up of reliable manufacturing processes. One difficulty with many OoCs is how to scale up system manufacturing to an industrial pace. Most early OoC designs are bespoke and fabricated in-house at the developers' institutions, where fabrication is limited by the cost and availability of both manufacturing equipment and personnel. Therefore, academic laboratories should focus on early quality control of the chips produced in-house to ensure reliability and reproducibility before scale-up can occur. This means careful compilation of standard operating procedures for chip design and creation, and designing clear quality control procedures that can be easily followed at other laboratories or manufacturers. Since most academic laboratories are not equipped to scale up production, the creation of spin-off or start-up companies or the formation of partnerships with manufacturing firms to mass-produce chips is necessary. At this stage, it would be extremely useful for all manufacturers to conform to Good Manufacturing Practice guidelines (see Related links) such as those issued by the FDA, which cover issues including equipment verification, process validation, sanitation and cleanliness of manufacturing facilities, and appropriate training of personnel. While this guidance is to ensure the safety and reliability of manufacturing processes for foods, drugs and devices for medical use, and is therefore not necessary for OoC manufacturing, it would still provide excellent standards for reliability of chip production across all fields and help to broadly increase confidence in the systems. To increase end-user confidence in the reliability and fidelity of mass-produced platforms, additional considerations should be taken that all biological assays are created on chips under good laboratory practice, as this is critical for preclinical toxicology testing and has been identified as a major reason for high drug development attrition rates[148]. In addition, there is a need for independent 'qualification' laboratories to test OoCs and their use with available cell types, much like the NCATS Tissue Chip Testing Centers (see earlier) or the European Union Reference Laboratory for Alternatives to Animal Testing (see Related links).

Onboarding versus outsourcing. Owing to the expense and complication of technology transfer for some OoCs, developers may face the decision between supplying a commercial product for purchase to be used independently in a customer's laboratory and offering services through a CRO to OoC consumers. If researchers decide to commercialize their OoC platforms, technology transfer and onboarding processes should be seamless, reliable and standardized for every customer. Meanwhile, retaining the personnel, infrastructure and resources necessary for OoC use within a CRO-based service means customers should expect high standards of the research produced. However, the flexibility and adaptation of the chips for specific contexts of use may be limited because CROs may not offer particular assays or services. As this burgeoning field is still young, many developers and companies are choosing to adopt aspects of both business models. Some offer OoC devices that can be onboarded relatively easily but may need specialized equipment and/or extensive technical support. Other CROs perform experiments in-house in collaboration with academic or industry researchers to help advance continuing research and development of the system.

Managing expectations. While the potential of OoCs is exciting, the technology is at an early stage, so providing realistic caveats and limitations to potential consumers is critical to avoid overselling its current capabilities. Some challenges faced within the field may be resolved over the next decade or so — issues with cell sourcing will continue to be addressed as the stem cell field matures, for example. Other limitations may take longer to resolve — for example, reduction and refinement of animal use are laudable and achievable aims and are within the realm of possibility already, but full replacement of animals in drug development is generally seen as unlikely in the near future.

One approach to managing expectations has been used by government funding agencies in the USA, where creating partnerships between research and regulatory agencies, such as the NIH and the FDA, over the past decade has allowed regulators access to OoC developers and their unpublished data to help inform system development. Conversely, it has enabled researchers to design useful platforms to provide data for regulatory assessment. This has led to familiarity of the technology among the regulatory community in the USA, which ultimately can help pave the way for OoC data inclusion in Investigational New Drug (IND) and New Drug Application (NDA) packages in the future.

Validating organs-on-chips

As OoCs continue along a path towards widespread commercialization, validation must be considered. Importantly, the term 'validation' means different things to various stakeholders, but could be considered as involving three stages or principles[149]. First, physiological validation could be defined in the context of 'analytical performance', including addressing features such as sensitivity, specificity and precision (essentially reproducibility). This validation step is necessary to create a tissue chip that appropriately and reliably mimics the tissue of interest and responds in relevant ways to compounds of known action or toxicity, and it should be performed by OoC developers. Second, qualification

Investigational New Drug (IND). An application submitted to the US Food and Drug Administration to administer a novel drug to humans. The first step in the drug review process, which includes information on animal studies, manufacturing protocols and clinical and personnel protocols. Data gathered become part of the New Drug Application.

New Drug Application (NDA). An application submitted to the US Food and Drug Administration requesting permission to sell and market a drug in the USA. Information submitted includes data from the Investigational New Drug and is reviewed for safety and efficacy, benefit versus risks, appropriate labelling information, and manufacturing and processing methods.

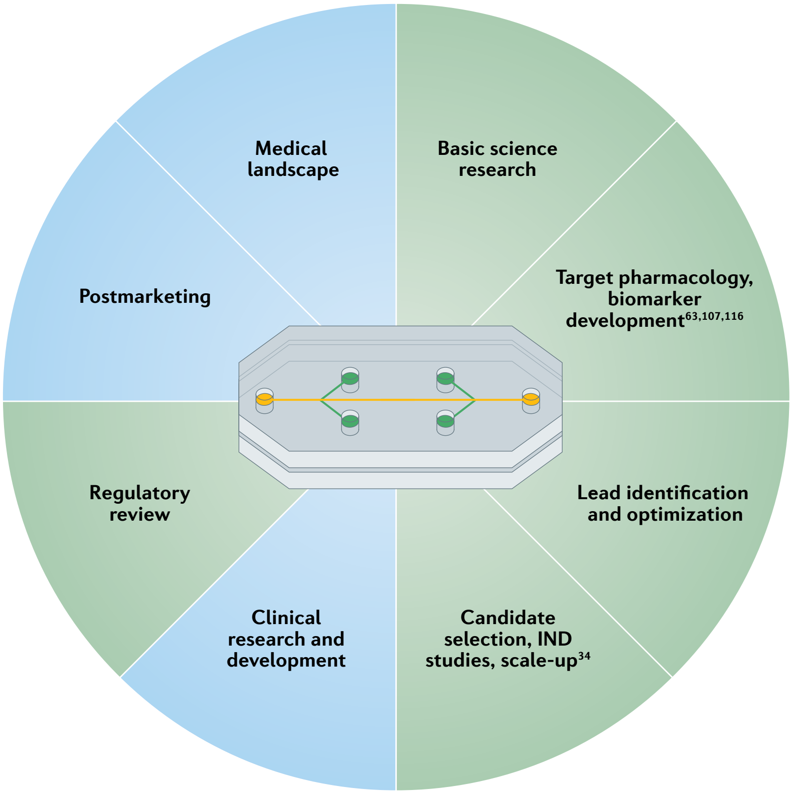

Fig. 3 | **Utility of OoCs in a variety of stages of drug development.** Drug development is a dynamic environment for data feedforward and feedback between multiple stages and processes, being described as a 'dynamic map'[158]. These dynamic maps provide a framework for understanding modern drug development and include activities and processes such as lead identification, clinical research and development, and regulatory review. Organs-on-chips (OoCs) can be informative in a number of these 'neighbourhoods'. In this schematic of an OoC surrounded by multiple stages and processes of drug development, green components represent the known current or shortly predicted use of OoCs and blue components represent the possible and predicted utility. Many OoCs are currently at the 'basic science research stage'. Use of OoCs in the 'medical landscape' stage includes use for precision medicine and patient-specific treatments. 'Clinical research and development' use would include patient subgroup stratification and projects under the NIH Clinical Trials on a Chip programme, as an example. 'Regulatory review' refers to Investigational New Drug (IND) and New Drug Application data. 'Postmarketing' refers to adverse drug reaction reporting and drug repurposing efforts. References are included for examples of OoC use in these areas. Figure adapted with permission from REF.[159], NIH.

or validation to show biological in vivo relevance should come next, although there is debate in the field as to whether animal or human responses should be used for this stage. Animal responses are broadly used in current drug development, which supports the argument that they should be the gold standard for OoC responses to be compared against. Conversely, predicting human responses is the aim for the field, which supports the focus on the generation of human responses on OoCs. Reproducibility and setting the standards for qualification currently fall under the remit of, for example, the NCATS Tissue Chip Testing Centers. The third stage, industrial validation, or OoC adoption by industry and regulatory agencies, will involve the generation of data from proprietary compounds and submission of those data to regulatory agencies. All of these stages of validation are currently under way. In the USA, the FDA has also partnered with a number of OoC companies to get hands-on experience with OoC data, as it expects data of this type to be submitted to it soon.

Taken together, the three stages/principles of validation/qualification described above will help address international guidelines for novel methods, for example the Organisation for Economic Co-operation and Development (OECD) "Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment" (see Related links) These guidelines describe necessary assay details for validation such as the rationale, the end points and limitations, protocols, variability, performance with reference and known chemicals, and comparisons with existing assays. Importantly, the OECD guidelines also state that data supporting the validity of the method must be available for review. To address this need for all stakeholders, the NIH's NCATS also funds the Microphysiological Systems (MPS) database, which integrates all the data from the NCATS Tissue Chip Testing Centers as well as data from a number of other NIH-funded developers, FDA users and commercial OoC suppliers. This centralized database acts as a public repository for a broad range of OoC data and will prove useful for developers, industry and regulatory bodies over the coming years, with a recent report highlighting functionality for data visualization, interstudy and intrastudy reproducibility and power analysis calculations[150].

Additionally, underpinning the needs of the aforementioned validatory steps, the accurate standardization of methods used for generating empirical data should be considered. The term 'standardization' brings new challenges with respect to what 'standardization' means for technical, analytical or biological aspects of OoCs. So, 'performance standards' should be established for the analytical validation and biological qualification of OoCs. To this end, the deposition of technical, analytical and biological data into the MPS database will help set some of the standards, reducing the need for users to develop their own methods, assays and analytical methods. At the same time, many US government-funded researchers are working with regulatory and industrial end users to evaluate what should be considered accepted metrics that are translatable to other laboratories and applications.

Emerging opportunities and prospects

There are multiple stages at which OoC platforms could be implemented in drug discovery and development, and the platform type may differ depending on the stage (see FIG. 3). High-throughput plate-based OoCs with relatively simplistic (but cheap and fast to produce) tissue constructs could prove useful for target identification, lead selection and lead optimization. Low-throughput to medium-throughput OoC platforms that model more complex tissue–tissue or organ–organ interactions could be more useful for preclinical single-organ or double-organ toxicity and efficacy studies. Multi-organ systems — while perhaps the most complex and expensive to develop — offer promise for reducing the need for animal studies and for use in parallel with phase I and phase II clinical trials. Finally, OoC platforms from patient stem cell-derived sources could be used during later clinical trial phases (III and IV) as well, for in vitro therapeutic testing before in vivo administration or for concurrent monitoring of approved therapeutics. Ultimately, the potential safety and efficacy of a drug or drug candidate could be evaluated with OoCs in generic, or even individualized, human platforms, giving 'first-in-human' testing a new connotation.

Coupling OoC technology with techniques such as gene editing[151] (particularly when a series of disease-relevant mutations are introduced onto a single genetic background) offers a powerful way to increase the predictive power of these tools further in disease modelling and toxicology. We also see opportunities to discover and validate clinically translatable biomarkers by creating datasets to correlate in vitro OoC readouts with clinical outcome measures. For example, use of OoCs to produce 'omics'-based (and even real-time) readouts could promote the identification and evaluation of appropriate end points surrogate to those in the clinic, which could provide valid and reliable measures of change in human participants. These end points and readouts could be quantified and assessed for clinical benefit and compared with traditional enzymatic, biochemical or histopathological assays, and could also offer ways to assess both short-term and long-term clinical changes. Ultimately, the use of OoC readouts detailing changes in molecular signatures that have been validated against traditional methods and demonstrated clinical relevance could become common practice in drug development.

To help smooth the adoption and implementation of OoCs in the drug development process, continued engagement and discussions with OoC developers and end users is critical, as is engaging with regulatory bodies. A 2017 report predicted that the global OoC market could grow by 38% per year to become a US$117 million per year industry in 2022 (based on market analysis by Yole Développement) — with the potential to become a multibillion dollar industry. In support of this predicted growth and the utility of OoCs at various stages of drug development, a recent analysis predicted up to a 26% reduction in research and development costs in the pharmaceutical industry by adoption of OoC technology[152], and it is anticipated that OoC data will be included in IND and NDA submissions to the FDA soon.

There is optimism that OoC systems may one day outperform traditional models, making the understanding of human diseases and development of drugs to treat them more rapid, efficient and cost-effective, and in so doing replace, reduce and refine (the '3Rs') the use of laboratory animals. Nevertheless, much work remains to address the challenges discussed in this Review, and thereby determine and realize the potential of this technology. According to the 2018 Gartner report (see Related links) on the hype cycle of emerging technologies, OoCs (referred to as 'biochips' in that report) are now in the 'peak of inflated expectations' phase. Disillusionment and a stall in progress often occur after this phase because the technology fails to live up to the preliminary, and often inflated, expectations, before the field recovers and productivity resumes with more modest expectations. Therefore, the aim for emerging technologies is to reach this productive plateau as quickly as possible, when 20–30% of the potential audience has adopted the innovation. Right now, this is estimated to be 5–10 years for OoCs. It will take the coordinated global efforts of the OoC community to help this technology reach that potential global audience and ultimately help transform science, medicine and patients' lives.

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Author contributions

L.A.L. wrote and edited the manuscript and created the figures. C.M., B.R.B., D.A.T. and C.P.A. reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

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