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Review article

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Integrating organoids and organ-on-a-chip devices

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Abstract

Organoids and organs-on-chips are two rapidly emerging 3D cell culture techniques that aim to bridge the gap between in vitro 2D cultures and animal models to enable clinically relevant drug discovery and model human diseases. Despite their similar goals, they use different approaches and exhibit varying requirements for implementation. Integrative approaches promise to provide improved cellular fidelity in the format of a device that can control the geometry of the organoid and provide flow, mechanical and electrical stimuli. In this Review, we discuss recent integrative approaches in the areas of intestine, kidney, lung, liver, pancreas, brain, retina, heart and tumour. We start by defining the two fields and describe how they emerged from the fields of tissue engineering, regenerative medicine and stem cells. We compare the scales at which the two methods operate and briefly describe their achievements, followed by studies integrating organoids and organ-on-a-chip devices. Finally, we define implementation limitations and requirements for translation of the integrated devices, including determining the differentiation stage at which an organoid should be placed into an organ-on-a-chip device, providing perfusable vasculature within the organoid and overcoming limitations of cell line and batch-to-batch variability.

Sections

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Key points

- Organoids and organs-on-chips (OoCs) aim to improve drug testing and disease modelling, but integration examples are still scarce.
- The benefits of integration include organ-specific cellular hierarchy and structural fidelity; microscopic features from OoCs guiding tissue morphological formation; better reproducibility and scale-up capacities; and biocompatible built-in sensors for in situ functional readouts and industrially compatible culture formats.
- A key challenge is vascularizing organoids with tissue-specific endothelial cells and aligning different cell types in organoids with appropriate flow in scalable, integrated devices.
- In parallel, advances in computer vision and deep learning will be needed to enhance data processing and analysis. Addressing cell line variability and establishing validation criteria for OoC–organoid integrated devices is critical for commercial and translational success.

Introduction

For each successful drug, a large portion of molecules in clinical trials fail due to efficacy (57%), safety (17%) or financial complications (22%)[1–3]. Models that better mimic human physiology could reduce these failures. Animal models, largely inbred, provide complex physiology and immune responses but face challenges in replicating human-specific responses and genetic diversity. Moreover, they are expensive and their use is ethically debated. Advances in stem cell technology now enable testing on human cells and tissues, which could revolutionize preclinical drug screening (Box 1).

2D cell cultures, in which cells are cultured on flat plastic or glass surfaces, have considerably advanced our understanding of fundamental biology and enabled assay development. It is often the simplest and most cost-effective system that can recapitulate physiological responses (such as compound-induced cell death). Moreover, owing to the ease of implementation and compatibility with high-throughput screening, it is commonly the first choice in the industry. Yet 2D cell cultures might not accurately reproduce the complex structure and physiology of native human tissues. Artificial 2D conditions and high substrate stiffness can alter cell morphology and gene and protein expression[4,5], which could ultimately impact drug responses[6].

In 3D cell cultures, cells are surrounded by other cells and the extracellular matrix (ECM), as they are in the body. Techniques for 3D culture include spheroids, organoids, tissue engineering relying on hydrogels or polymeric scaffolds, and cultivation in customized organ-on-a-chip (OoC) devices. Owing to the more physiological arrangement of cells, 3D cultures might better recapitulate in vivo cellular interactions, morphology, and gene and protein expression. Despite these advantages, 3D cultures are often more complex, requiring specialized equipment and expertise, making them more costly and technically challenging for assay design.

In this Review, we start by defining both organoids and OoCs, emphasizing their physical scale and discussing their relation to the techniques and concepts pioneered in the fields of tissue engineering and regenerative medicine. We then emphasize the motivation behind the integrated approaches, specifically the promise to create a superior culture system with an in vivo-like cellular fidelity, flow control, biophysical stimuli and sensor integration in a single system. Finally, we provide recommendations for new researchers and suggestions to experienced ones for how to move the field towards better translatability.

Organoids and OoCs

Organoids

Organoids are defined as self-organized structures, most often arising from human pluripotent or adult stem cells undergoing expansion, in vivo-like differentiation and morphogenesis[7,8] (Fig. 1). They contain multiple cell types and cytoarchitectural and functional features that resemble specific organ regions[9]. Some organoids (such as intestinal or kidney organoids) are histologically indistinguishable from the native organs[10]. Important exceptions include tumour organoids, which emerge from cells isolated from the primary cancer tissue and can divide and produce a high level of self-organization. Moreover, differentiated cells, such as cholangiocytes, can acquire cellular plasticity and clonally expand as self-renewing liver organoids that retain their differentiation capacity into both hepatocytes and ductal cells[11]. Cellular plasticity, self-organization and the presence of multiple cell types are also important hallmarks of these organoids. All other models where already differentiated cells or cell lines are forced into spherical structures are termed spheroids.

Modern organoid research builds on important historical advances. In the early twentieth century, sponge cells were shown to generate organisms in vitro[12]. Later, various organs were regenerated from chick embryo cells[13]. In the 1980s, mouse pluripotent stem cells, followed by human embryonic stem (hES) cells, were identified for the study of embryogenesis[14,15]. The 2007 creation of human induced pluripotent stem (hiPS) cells addressed ethical concerns associated with hES cells[16]. Yet many organoids can be derived from multipotent stem cells present in the adult tissues[10].

Recognizing the need to mimic the in vivo environment led to the 3D culture of polarized cortical tissues[17]. Precise spatio-temporal control of Wnt, bone morphogenetic protein (BMP), sonic hedgehog (SHH), fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) signalling pathways ultimately led to the creation of region-specific organoids and their assemblies to study diseases such as autism and microcephaly[18,19]. The organoid field then rapidly expanded to model retina[20], liver[21], kidney[22], pancreas[23], lung[24] and prostate[25]. Advanced biomaterials improved temporal patterning, enhancing biomimicry and functional maturation in organoids[26,27].

Despite the advantages of organoids in achieving morphogenesis and sophisticated multi-cellular organization not amenable in 2D, they have some limitations. They lack systemic interactions with other organs and tissue compartments. Functions such as a perfusable vasculature and the immune system are often absent. Although organoids can be cultured in vitro for months, their growing size often results in a necrotic core[28], limiting their advanced functional development.

To overcome some of these limitations, assembloids were developed, which are self-organizing cellular systems resulting from the combination of two or more different types of organoids[9]. For example, fusing cerebral cortex or hindbrain or spinal cord organoids with human skeletal muscle spheroids results in a cortico-motor assembloid[29]. Similarly, fusing brain organoids with different regional identities results in the migration of interneurons from ventral to dorsal forebrain regions[30].

Organ-on-a-chip

An OoC is an engineered or microfabricated cultivation system that supports cell assembly into tissue-like structures and enables measurements of the functional hallmarks of the mimicked organ (Fig. 1). Without reproducing the entire organ, OoC systems offer more accurate 3D cell culturing, enabling the simulation of the physiological responses of one or multiple tissues. Moreover, several tissues making up one organ can be combined in an OoC to reproduce a defined functional hallmark of that organ, for example, transport across the epithelial or endothelial barriers to measure permeability. The term OoC was first introduced in 2010 in a lung-on-a-chip platform[31]; however, cell cultivation within microfluidic devices was already established in the 1990s and early 2000s, when a lung, liver and fat platform connected with microfluidic channels was used for modelling of compound toxicity[32]. Since then, the field expanded rapidly as various platforms were developed to mimic vasculature[33], liver[34], heart[35,36], gut[37], kidney[38], brain[39] and bone[40].

Although classical OoC devices contain flow, it is not a prerequisite for an OoC system. Instead, all systems that enable precise control of a microtissue structure[36,41] or allow the application of physical stimuli, such as electrical stimulation for cardiac systems[35], shear stress for vascular systems[42] or mechanical strain for skeletal muscle systems[43], are considered to be OoCs (Fig. 1).

OoC platforms exhibit a set of common characteristics, including the incorporation of multiple cell types, such as vascular, stromal, parenchymal and immune cells, to better mimic the physiological heterogeneity in the native tissue. Another feature is the presence of a membrane or a pillar array to facilitate the transport of nutrients and oxygen, structures for cell aggregate trapping, compartmentalization within the chip, or geometry control via tissue fixation for cellular alignment and multiaxial stretching. Finally, the establishment of distinct cellular compartments should recapitulate key organ functions (Fig. 1).

Moreover, OoC systems might enable combining multiple tissues within a single device to provide insights into organ-level interactions and physiology in a controlled environment, which is often not possible in conventional 2D cell cultures. The controlled microenvironment of OoC also enables the investigation of subtle changes, often not possible in animal models. Despite their complexity, these systems may not always fully capture the systemic responses to treatment. Additionally, they are often expensive to implement and require specialized expertise.

Size scale and human organ fidelity

Organoids, assembloids and OoCs are advanced 3D cell culture technologies that can model certain biological processes of the target tissues. However, they still lack appropriate vascularization, routine evaluation of pharmacokinetics and pharmacodynamics, identification of drug tolerization mechanisms and off-target effects[44]. None of the systems reproduce the entire organ, and they operate on the scale of hundreds of micrometres to ~1 cm (Fig. 1). Most often, the tissue component that is being reproduced is responsible for a critical function of that organ. For example, heart-on-a-chip devices would reproduce a contractile force that is a primary function of the cardiac muscle by providing bundles of cardiac muscle cells[35]. However, they do not reproduce the four-chambered heart and not even the ventricular wall itself, consisting of the endocardium, myocardium and epicardium.

Tissue engineering and regenerative medicine

OoCs and organoids are in vitro approaches that can be used for tissue engineering and regenerative medicine applications. Tissue engineering is defined as an integrated use of cells, biomaterial scaffolds and bioreactors to create tissues that can probe disease aetiology, drug efficacy, and developmental mechanisms and replace or augment the structure of native tissues. Similar principles in OoCs exist, except that OoC approaches are focused on a smaller scale[45] (Fig. 1).

Box 1 | Translational considerations

Organoids and organ-on-chip (OoC) approaches can augment 2D culture where improved physiological fidelity is required. Both fields have witnessed substantial commercialization efforts[200]; however, pharmaceutical and biotech companies have yet to switch from animal models to 3D tissue systems. There are two main drivers for this lack of adoption: the first being an animal study-centric regulatory environment. The recent FDA modernization act signals broader support for non-animal pathways (such as organoids and OoCs) in regulatory document submissions[192]. Despite pharmaceutical companies being able to use the non-conventional models even prior to the passage of the act, the true obstacle was that these advanced in vitro systems were not fully qualified to be predictive of human drug responses, requiring validation of the readouts equivalent to those of animal models that were traditionally accepted by the regulatory agencies. Additionally, not all organs have yet been modelled, which further deters pharmaceutical companies from making decisions based on these non-traditional systems[201]. The second likely driver is related to the overall difficulty in setting up 3D cell culture systems in-house. Whereas the reagents, plates, equipment and trained personnel are available to easily run either 2D or animal studies, 3D studies often suffer from a lack of existing infrastructure in pharmaceutical and biotech laboratories, low availability of trained staff, 3D assay variability as a result of the intrinsic cell line variability, lack of regulatory approved benchmark strategies, and the lack of scalability of current OoC systems. Setting up studies with organoids is currently considered easier than with OoC systems due to the hardware requirement of the latter. Large pharmaceutical or biotech companies (such as Roche or Genentech) are now starting to develop their own advanced 3D tissues in-house, further incentivizing both organoid and OoC companies, as well as academic researchers, to make their models more user-friendly.

Despite faster artificial intelligence-mediated identification of a number of exciting molecules, testing these in animal models will not result in an accelerated discovery cycle. The next big frontier may be combining OoC or organoids and artificial intelligence approaches, a trend evident from recent mergers, such as Valo Health, an artificial intelligence company, acquiring the heart-on-a-chip company TARA Biosystems, or companies such as Vivodyne, Tissue Dynamics and Quris combining OoCs and artificial intelligence in-house. Similarly, BICO is integrating a number of companies under one umbrella to create a bioconvergence hub.

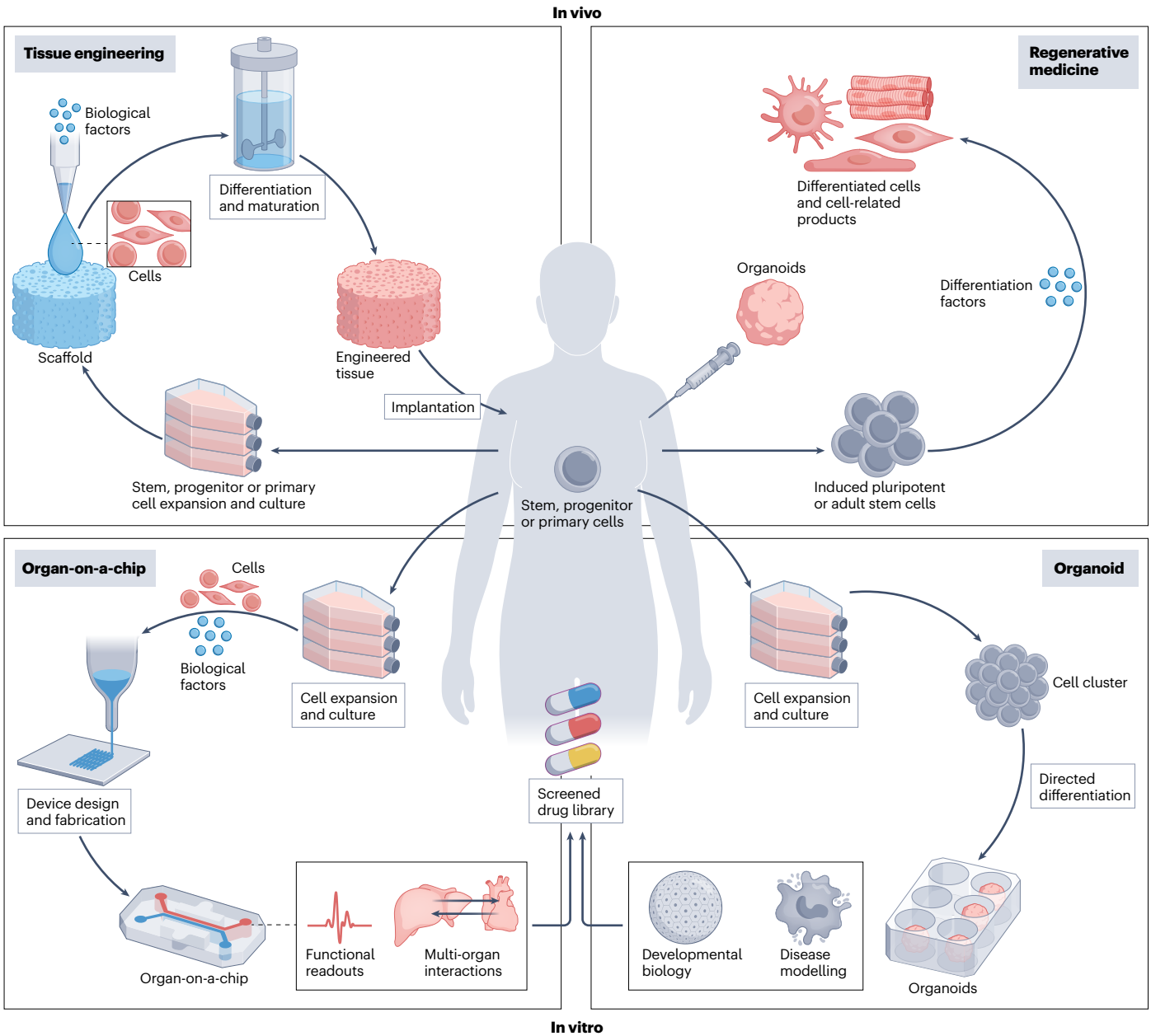


Fig. 1 | Main approaches in organoids and OoC systems. Organoids originate from single cells or small clusters of adult or pluripotent stem cells that are cultured in vitro and undergo an in vivo-like differentiation to organ-specific tissue structures. The differentiation process recapitulates embryogenesis, passes through the appropriate germ layers (endoderm, ectoderm and mesoderm) and adapts the regenerative medicine approach. Organoids can also be derived by self-organization of primary cells. Organs-on-chips (OoCs) are an engineering approach to combine cells, scaffolds and topographical guidance to derive miniature functional tissue models with desired tissue organization. By integrating organoids with OoC technology, researchers can address some of the key challenges in both fields, including tissue vascularization and generation of a high-fidelity parenchymal–vascular interface incorporating multiple cell types, multi-organ communication via multiple connected organ-specific chips, and integrated sensors and biological stimuli for tissue maturation and in situ functional assessments.

Regenerative medicine focuses on using either pluripotent or adult human stem cells, their progeny and related technologies (such as gene editing) to replace, regenerate and functionally restore tissues and organs damaged due to age, disease, accidents or congenital defects[46]. Therapeutic use of organoids via transplantation to regenerate organs, such as the liver and the intestine, has also been proposed[47]. Both regenerative medicine and organoid research[17,18,30,48] use directed differentiation protocols that rely on the application of cytokines designed to activate pathways responsible for organogenesis during development to enable highly specific cell differentiation (Fig. 1).

Motivation for integration

At a high level, integration means synergistically combining the advantages and techniques from each field into a unified system to overcome the respective challenges experienced in each field. The well-defined geometry and microfeatures acquired from OoC microfabrication and hydrogel moulding can help guide and control the structure during organoid development to improve consistency and organoid morphological maturation. OoCs could facilitate integration between the organoid and the perfusable vasculature and enable in situ functional readouts with integrated sensors. Moreover, organoids can endow OoCs with sophisticated cellular composition by providing organ-specific multi-lineage cellular populations, either through direct culture of organoids in OoC devices or by digesting the cells out of organoids and applying them in OoC devices. These advantages are evident from pioneering studies targeting organs such as the colon[49], kidney, retina and heart, or the neurovascular interface or breast cancer (Fig. 2). Yet more experiments are needed to achieve the full potential of integration.

An OoC device is often a membrane positioned in a microfluidic channel; on one side of the membrane, epithelial cells are cultivated and the other side of the membrane houses endothelial cells (Fig. 3). These setups provide structural support of cells and vascular lumens, yet sometimes, with limited biological relevance. By contrast, organoids (such as kidney[22]) contain multitudes of kidney-specific cells and structures, thereby better representing human tissues in terms of cellular fidelity and structural relevance (Fig. 3).

Nonetheless, OoC approaches enable the control and driving of flow by carefully tuning inputs and outputs while incorporating physical stimuli (Fig. 3). OoC devices can also conveniently integrate various sensors, such as oxygen probes, displacement and force sensors, or electrodes for the recording of extracellular field potentials[50,51] (Fig. 3). Although sensors have recently been integrated into organoids[52,53], they are less mature than the advanced sensing capabilities in OoCs. Moreover, OoC device cultivation reproducibility is an advantage over organoids that are often limited by the necrotic core formation[28] and batch-to-batch variability (Fig. 3).

Organoids are frequently cultured in suspension or embedded within an ECM, enabling self-organization and assembly. Their integration within OoC platforms necessitates the containment of their growth within the defined boundaries of microstructures, which may influence emergent properties and cell lineage determination and introduce culture artefacts. Organoids are also more compatible with the current pharmaceutical pipeline and high-content screening machines (Fig. 3). It is generally easier to perform fluorescence imaging of organoids than of cells in OoC devices, with imaging in the integrated systems expected to be even more challenging. Yet simply pipetting drugs on top of organoids fails to capture the distribution of the drug across the vascular barrier, diffusion through the stromal space and partitioning in the body. Biomimetic vasculature remains a challenge in the field; OoCs typically incorporate predefined vessel networks (sometimes with limited biological relevance), whereas organoids often self-assemble and develop into micrometre–centimetre-scale, multi-cell-type organ mimetics without flow control. Integration would be a clear advantage.

In terms of outputs, both organoids and OoCs are amenable to standard techniques such as immunofluorescence staining and microscopy, as well as '-omics' analyses to determine gene and protein expression, metabolic signatures, and the activity of various enzymes. Using electrodes, electrical field potentials can be recorded in both organoids and OoCs[36,50,52,53]. In conjunction with reporter dyes, other measurements, such as Ca^{2+} transients, can be obtained[35]. Owing to the ability to control flow, OoC approaches can determine the function of barriers with both transepithelial electrical resistance measurements and by studying the transport of molecules, viruses and bacteria over epithelial or endothelial interfaces. Defined positions of the physiological barriers within OoC devices facilitate visualization of the species crossing the interface. Functional measurements, such as contraction force and impulse propagation, are also better measured in OoC systems due to their defined dimensions (Fig. 3). Conversely, sophisticated readouts, such as single-cell RNA sequencing, are probably more meaningful when performed in organoids, owing to the higher cellular fidelity.

Advanced integrated systems

Integrating organoids and OoCs implies that a higher level of model complexity can be achieved (Box 2). We specifically discuss how this integration represents an advantage over what could be obtained using a single approach (Table 1). Ultimately, the model of choice depends on the biological question and, in some cases, either organoids or OoCs alone might be able to provide the answer.

Small and large intestine

Although intestinal organoids are complex and retain the tissue architecture of their native counterparts, including the preservation of intestinal crypt, villi and cellular heterogeneity[54], they are still limited by a lack of vascularization, perfusion, peristaltic motion and the ability to introduce gut microbiota. These aspects have been captured by OoC technology[55]. Moreover, the availability of small and large intestinal organoids from biobanks and the commercialization of intestine organoid culture medium have accelerated their adoption in bioengineering labs, leading to integrated approaches.

For example, a self-assembled perfusable vasculature was wrapped around organoids in iFlow plates[49] (Fig. 2b and Table 1), enabling the modelling of immune cell recruitment during colon inflammation, which is difficult using a static organoid culture. Yet the luminal space of the organoids remained enclosed and inaccessible, thereby limiting studies of barrier function.

To overcome this issue, a perfusable device with an intestinal-specific vascular compartment and an epithelial compartment separated by a porous polydimethylsiloxane (PDMS) membrane was used with intestinal biopsy duodenum organoids[56], mimicking peristalsis motion by stretching (Table 1). The intestinal organoids were able to mimic villi-like structures, intestinal folds and intestine microenvironment similar to that of the native human intestine. The integrated system enabled integration of mechanical cues essential for villi formation and inducing peristaltic motion, which would not have been possible with conventional organoid culture. Furthermore, transcriptome analyses showed that the integrated model was able to better recapitulate intestinal physiology and expressed genes important in digestion, nutrient and drug transport, and led to intestinal immunity similar to that of native human intestine compared to duodenum organoids. Despite these advancements, the model lacked tubular geometry.

Using a similar OoC device, a colon-on-a-chip model with a mucosal bilayer was developed[57] (Table 1). The primary colonic epithelial cell-derived mucus bilayer mimicked human colon in thickness and the composition of mature goblet cells, responsible for mucus production. The exposed organoid epithelium enabled studies of nutrient and drug transport, yet the tubular geometry of the intestine

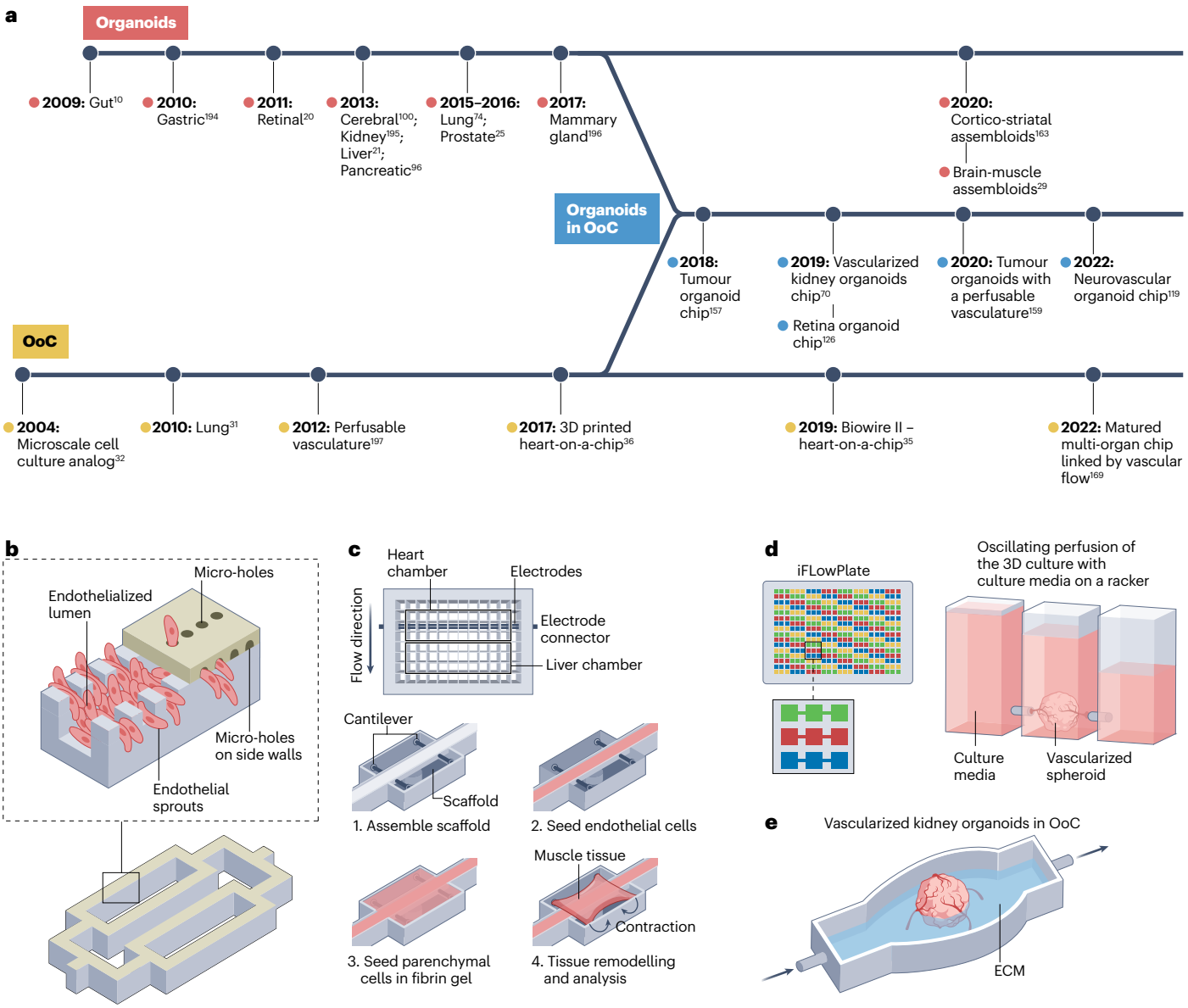


Fig. 2 | Timeline and examples of multi-well devices for integration of organoids and OoC devices. a, The fields of organoids and organ-on-a-chip (OoC) engineering pursued similar goals of developing improved models for drug discovery and disease modelling separately, with examples of integrated studies recently emerging. Since gut organoids[10] in 2009, there has been a boom in the development of organ-specific organoids, including gastric[194], retinal[20], cerebral[100], kidney[195], liver[21], pancreas[96], lung[74], prostate[25], mammary gland[196] and assembloids[9,29]. The OoC field instead started with the first microscale cell culture system in 2004 (ref. 32) and lung-on-a-chip[31] in 2010, followed by vasculature[197], heart[35,36] and multi-organ system[169] connected by common circulation to increase the biological complexity. The integration of organoids with OoC devices further improved the physiological relevance and tissue functions, demonstrated by tumour organoids-on-a-chip[157,159], retina organoid-on-a-chip[126], neurovascular organoid-on-a-chip[119] and kidney organoid-on-a-chip[70]. Examples of perfusable multi-well platforms for OoC modelling and organoid integration. **b**, Branching AngioChip facilitates the formation of a multi-hierarchical vessel network[176]. **c**, 96-well plate-based platform with AngioTube vascular scaffold created by 3D stamping[159]. **d**, IFlowPlate cultures 128 independent units by connecting three wells via microfabricated conduits on 384 well plates. The platform can harbour single cells or organoid culture with continuous perfusion[49]. **e**, Kidney organoids with in-growth of perfusable vasculature on the microfluidic OoC platform[70]. ECM, extracellular matrix. Part **b** adapted from ref. 176, Springer Nature Limited. Part **c** adapted with permission from ref. 140, Wiley. Part **d** reprinted with permission from ref. 49, Wiley. Part **e** reprinted with permission from ref. 70, Springer Nature Limited.

was lacking. This limitation was overcome by culturing intestinal stem cells in crypt-like topographical patterns in a hydrogel[58] and introducing luminal perfusion[59]. The integrated models were effective in modelling radiation injury and chronic parasite infection, which was not possible in standard organoid culture due to the lack of access to the luminal space[59].

The intestine is notable for many examples of integrative studies; however, further improvements can be made. The integration of gastrointestinal smooth muscle cells with intestine organoid-on-a-chip systems has not yet been achieved. This is crucial for replication of peristaltic movement and muscle hyperplasia in diseases. Other non-gastric cell types, including mesenchymal, neural and immune cells, are also absent. Their inclusion requires meticulous efforts for media optimization.

Kidney

The basic functional unit of a kidney — a nephron — is further divided into subunits, including the glomerulus, proximal tubule, loop of Henle, distal tubule and collecting duct[60]. Proximal tubule or glomerulus have been engineered separately by spatially arranging the primary or (conditionally) immortalized tubular epithelial cells or podocytes in OoC devices to resemble their native configurations with respect to endothelial cells[61–66]. The devices used tubular hydrogels or porous membranes to capture the barrier function; however, the few cell types in these devices do not recapitulate a full nephron.

By contrast, in hES cell-derived or induced pluripotent stem (iPS) cell-derived kidney organoids, segmented nephron-like structures were organized continuously, resembling the in vivo nephron containing podocytes, proximal tubule, distal tubule and, even, collecting duct cells[22,67–69]. Although vascular markers were present, kidney organoids lacked robust vasculature, could not capture barrier function and the cells were immature relative to the adult kidney[22].

The vascularization and maturation aspects were addressed by growing organoids in 3D-printed perfusable chips[70,71] (Table 1 and Fig. 2c). Transepithelial transport function was also recapitulated in tubular epithelial organoids from human urine or biopsy samples grown in commercial OrganoPlates with ECM[72]. Seeding tubular epithelial cells isolated from kidney organoids in cylindrical channels of the OoC device resulted in enhanced drug uptake relative to that of control chips with immortalized proximal tubule epithelial cells[73].

Although the integrated organoid and OoC approach improved vascularization, maturation and modelling fidelity of specific nephron sub-structures, modelling the entire nephron is still a challenge. OoCs could provide the desired spatial arrangements for different renal cell types from kidney organoids to control flow and transport properties in different segments.

Lung

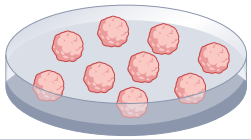
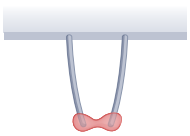
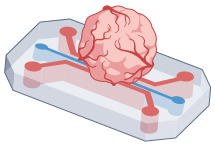
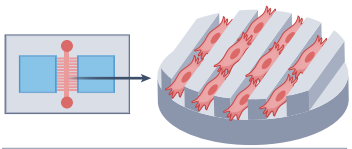
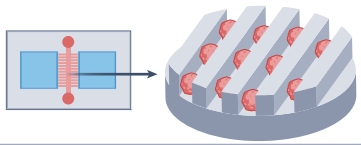
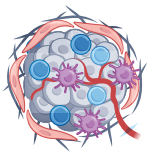
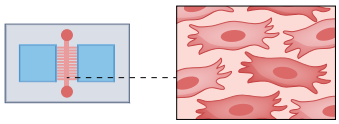
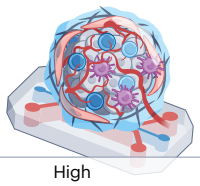
The lung is comprised of branched airway ducts leading to terminal alveolar sacs, which together are responsible for gas exchange. iPS cell-derived foregut spheroids embedded in Matrigel were induced into a lung lineage to form human lung organoids consisting of proximal airway-like structures by modulating FGF and Hedgehog signalling[74]. After 2 months, the organoids contained basal cells, immature ciliated cells, rare club cells and bipotent alveolar progenitors, yet without branching morphogenesis[74]. By manipulating BMP and Wnt signalling, branching structures were achieved in hES cell organoids after 170 days[75]. These examples highlight the importance of long-term culture for lung organoid differentiation. However, the resulting cells were still fetal-like, necessitating additional chemical and biomechanical factors to further enhance and accelerate lung organoid maturation. Adult stem cell-derived airway and nasal organoids have been established using lung progenitors from patients[76] and could be used to study SARS-CoV-2 infection[77]. Nevertheless, unlike hPSC-derived organoids, they are already fully committed to either proximal or distal lung lineages and no longer display branching morphogenesis.

Microfluidic-based, lung-on-a-chip models can reproduce a dynamic environment and mostly rely on cell lines or primary lung cells. Primary lung cells have some degree of differentiation potential but exhibit a finite number of passages, a challenge that can be addressed by using stem cell-derived organoids. The first lung-on-a-chip model recapitulated the alveolar–capillary interface by seeding a human alveolar epithelial cell line and human primary pulmonary microvascular endothelial cells on opposite sides of a porous PDMS membrane inside a microfluidic chip[31]. Stretching the support membrane via a vacuum was used to mimic the breathing motion[31,78]. In vivo, in addition to stretching, the airway epithelium is exposed to airflow-induced shear stress. Thus, an air–liquid interface culture provides a pseudostratified epithelium with cellular diversity and composition similar to in vivo lung[79]. Airflow further drives epithelial differentiation to obtain mature cilia and mucus-producing cells[80]. When self-assembled vasculature is incorporated with the airway epithelium in a 3D-printed perfusable device, the mucociliary differentiation is further improved[81]. Besides stretching and flow, the confinement of lung progenitors in 100-μm tubes promoted differentiation towards distal cells compared to 400-μm tubes or flat surfaces, suggesting that topography could be an important factor to consider in organoid cultures[82] (Table 1).

Lung organoids often possess supporting stromal cells that self-organize around the lung epithelium. Traditional membrane-based lung-on-a-chip devices neither readily accommodate stromal cells nor permit a mixed cell population to self-compartmentalize. In integration approaches, it might be important to moderate the use of engineered structures to avoid hindering the self-organization of organoids. Membrane-free lung-on-a-chip devices that rely on hydrogels could offer a more conducive environment for such integration. Another challenge lies in the timing of integration; for example, lung organoids, depending on their differentiation stage, favour distinct matrix coatings and exhibit varied proliferation rates. The seeding process in OoC devices typically imposes practical constraints on surface coating and post-seeding cell proliferation requirements. Therefore, optimizing the timing of integration becomes essential. For instance, cells with lower levels of differentiation tend to exhibit higher rates of proliferation, a trait beneficial for seeding in OoC systems. However, this cell population is often more varied, and previously established differentiation protocols may need adjustments to accommodate the new OoC environment. Conversely, cells at more advanced stages of differentiation display limited proliferative abilities, posing challenges in achieving a confluent barrier within the OoC system. Thus, optimizing the timing of integration becomes essential and would require close collaboration between bioengineers and stem cell biologists.

Liver

Liver functions, such as detoxication of blood, biosynthesis of plasma proteins, production of bile, metabolism and biotransformation, are enabled by multiple cells (sinusoidal endothelial cells, hepatocytes, Kupffer cells and stellate cells) that interact in concert to facilitate complex communication. Multiple isoforms of human cytochrome P450 (CYPs, CYP1A, CYP2C, CYP2D and CYP3A) show appreciable differences in enzymatic activity compared with common animal models (mouse, rat, dog, monkey); therefore, the extrapolation of drug toxicity based on animal data should be made with caution[83], which further complicates predictions of drug-induced liver injury in humans.

	Organoid	OoC	Integration of OoC and organoids
a Adaptation			
	Easy	Moderate	Hard
b Environmental control			
	Low	High	High
c Cellular fidelity			
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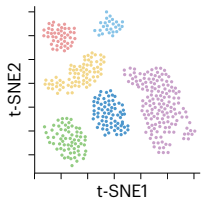
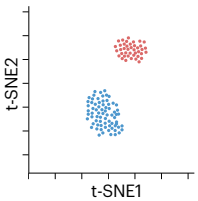
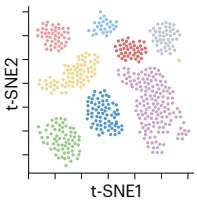
d In vivo benchmarking			
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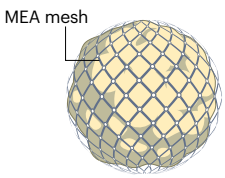
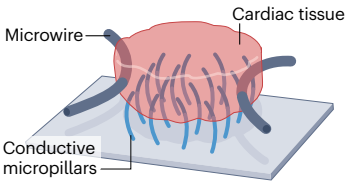
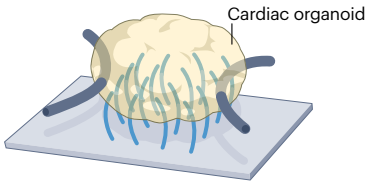
e Built-in readouts			
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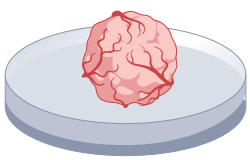
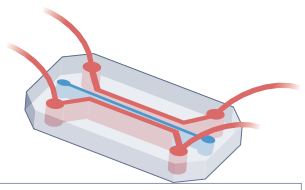
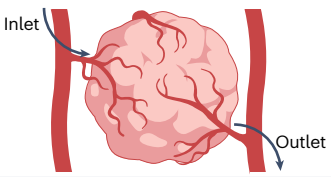
f Input/output flow			
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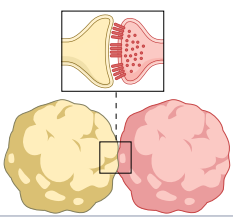
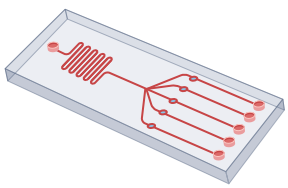
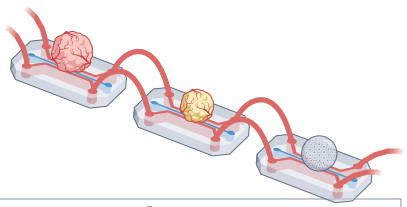
g Multi-tissue integration			
	Present	Present	Present

Fig. 3 | Advances and challenges in the integration of organoids and OoC systems. a, Organoids require less hardware and are easier to implement as opposed to organ-on-a-chip (OoC) devices and integrated systems. From left to right: cancer organoid cultured within hydrogel dome; cardiac-on-a-chip platform for culture and maturation of cardiomyocytes in vitro[198]; integrated organoid and OoC. **b**, OoC systems provide improved environmental control compared with organoids. Integrated systems enable the design of complex cellular microenvironments and hence create even more accurate models. **c**, Cellular fidelity is higher in organoids than in OoCs, owing to the multi-lineage differentiation that occurs in the former. Organoid-on-a-chip platforms provide a unique opportunity to achieve high-fidelity organoids with controlled microenvironments[126]. **d**, Next-generation data analysis, through machine learning and artificial intelligence algorithms, has been applied extensively to organoids, whereas OoC approaches are just starting to be equipped with such data analysis capabilities. The combination of high cellular fidelity of organoids with the controlled microenvironment of OoCs could better recapitulate the native tissue. **e**,**f**, Built-in sensor-based readouts or flow control are generally limited in organoids, whereas they are required for OoC devices. Integrated systems are expected to include sensing and flow control capabilities. **g**, Multi-organ integration is limited to assembloids largely due to the requirement for different culture media and the absence of barrier structures that would separate the organ compartments, whereas it is present in OoC devices. An integrated system further facilitates the interconnection of different types of organoids. MEA, microelectrode arrays. Part **a** adapted from ref. 198, Springer Nature Limited. Part **e** is adapted from ref. 199 CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Liver-on-a-chip systems usually consist of hydrogels encapsulating human cell lines, human primary cells or human iPS cell-derived hepatocytes behind a porous membrane that permits transport of metabolites and small proteins. Perfusable channels on the other side of the membrane protect hepatocytes from the detrimental effects of high shear stress, while enabling the presence of flow for enhanced mass transfer. Hepatocytes that grow in devices with the presence of flow not only produce more albumin, urea, metabolites and growth factors but also exhibit a more mature phenotype[84]. These systems have been used for liver disease modelling[84], as well as to study the role of liver metabolism in modulating drug effects[85] and animal species-specific drug responses[84]. Tissue aggregates have also been cultured in perfusable microenvironments to study inter-organ crosstalk, including islet–liver[86], liver–intestine–stomach[87], liver–lung[88], liver–T cell[89] and liver–heart[90] axes (Table 1).

Hepatocyte-containing iPS cell-derived liver organoids exhibit improved liver functions relative to those of static culture of organoids and 2D culture, as well as higher structural similarity to in vivo liver buds[21]. A gene expression analysis of liver organoids also demonstrated similarities to human fetal liver buds[21]. Integration of human liver progenitor-derived organoids into OoC systems improved albumin and CYP gene expression, cellular ultrastructure features, polarization, bile canaliculi formation and actin organization[91] relative to static culture of pre-differentiated organoids (Table 1).

The absence of functional vasculature is a major limitation for the advancement of faithful liver models. Liver sinusoidal endothelial cells (LSECs) are the most abundant non-parenchymal cell type in the liver, which maintain metabolic and immune homeostasis and actively contribute to disease pathology. Vascularized liver buds express higher levels of hepatic marker genes than non-vascularized ones do and achieve functional anastomosis within 48 h of implantation[21,92] (Table 1). Functional anastomosis can also be established using a microfluidic system, in which pre-vascularized liver spheroids are placed on top of a perfusable vascular bed[93]. Despite this progress, the heavy reliance on human umbilical vein endothelial cells (HUVECs) limits faithful capture of liver physiology, as LSECs contain fenestrae that mediate the passage of macromolecules, a feature that is missing when using HUVECs. With the emergence of LSEC differentiation protocols[94], HUVECs can ultimately be replaced.

Moreover, in vitro liver models should exhibit spatial heterogeneity along the liver sinusoid, that is, the biochemical gradients of oxygen, cytokines, nutrients and signalling events. Only a few microfluidic platforms have focused on reproducing this liver zonation to capture zonal drug toxicity[95]. Careful validation of the expression of CYP enzymes and transporters in hepatocytes in different zonations is recommended.

Pancreas

Pancreatic islets are clusters of endocrine cells composed of insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-releasing δ-cells, pancreatic polypeptide-releasing F cells and ghrelin-releasing ε-cells. Conventional 2D and 3D cultures often lack physiological functions of the pancreas, such as the control of ion channel activity due to shear stress and the regulation of insulin production by stretch-induced changes in cytoskeletal architecture, limitations that could be overcome via pancreatic organoid culture[96].

Modelling complex diseases, such as cystic fibrosis, in which mutant cystic fibrosis transmembrane conductance regulator channels in pancreatic ductal cells impact the production of the alkaline isotonic fluid necessary for digestion, requires more realistic models. A pancreas-on-a-chip with ductal organoids improved the crosstalk between pancreatic ductal epithelial and islet cells to study the relationship between cystic fibrosis and diabetes[97] (Table 1). For diabetes applications, quantifying insulin secretion is essential. Microfluidic platforms enable such functional assessments of islet organoids, including glucose response and insulin secretion, oxygen consumption, and calcium influx[98] (Table 1). The hydrophobic nature of PDMS, used in both of these studies, presents certain challenges due to its tendency to absorb small hydrophobic molecules (such as drugs), thereby complicating the accurate replication of physiological drug effects.

The generation of functional pancreatic islets requires highly controlled cell–cell communication, activation of transcription factors (such as PDX1 NKX6.1, NEUROD1, MAFA and PAX6) and blood flow[99]. Cultivation of pancreatic organoids in OoC devices may provide the necessary dynamic control to address these shortcomings.

Central nervous system

Brain. During embryonic development, spatio-temporal control of molecular events directs the rapid expansion and functional maturation of the human cortex[100,101]. Similarities in cell composition, zonal organization, gene expression and proteome between human cerebral organoids and human prenatal neocortex have been revealed[102,103]. Long-term culture of cerebral organoids can be achieved for late stages of neural development, including axon outgrowth and neuronal maturation[104]. Region-specific organoids[105], including forebrain (cortical[19], subpallium[30], hippocampal[106], thalamic[107] and hypothalamus[108]), midbrain[109] and hindbrain[110] organoids, have been

Box 2 | Technological transfer considerations

Beyond the scientific aspects, choosing a suitable business model will directly impact the path of technological transfer.

Service-based business model

This model is more feasible if there are large service contracts or co-development agreements already in place between the developer and at least one pharmaceutical company but require substantial investment in infrastructure and personnel. For an organ-on-chips developer that focuses on developing a product for just one tissue or disease type, this might be a better route considering the limited market size and the importance of generating validation data to gain credibility.

Product manufacturing is still critical in the service-based model because quality control needs to be in place at every stage of the data-generating process. Thus, engaging with manufacturers earlier to streamline device manufacturing is recommended. The cost of device manufacturing is usually a small fraction of the operating costs; therefore, there is room to explore more complex fabrication techniques and/or operational procedures (for example, cell seeding, tissue production, analysis) with well-trained personnel based on experimental needs. This can be an advantage compared with a product-based business model. For example, before its acquisition, TARA Biosystems offered drug testing and disease modelling services in the Biowire II induced pluripotent stem cell-based heart-on-a-chip platform. Axiosim and Ananda Devices offer testing services on precisely organized central and peripheral nerve cultures.

Product-based business model

Product manufacturing involves a substantial fraction of business development in this model; therefore, manufacturing costs and supply chain become paramount. To do so, product designs should be versatile and applicable to a broad range of tissues while remaining simple enough to be amenable to multiple industry-standard manufacturing techniques. Due to cost considerations, it is very likely that a product at the early stage of commercialization must be made using low-throughput methods, such as computer numerical control machining or 3D printing. Nonetheless, the design also needs to be compatible with high-throughput methods, such as injection moulding, at the later stage of commercialization.

Consumers are constantly balancing the trade-off between ease of use and the benefit offered by the product; therefore, the device must be user-friendly to reduce the learning curve. This factor alone will likely determine the speed of technology adoption. Working with a network of experienced users as early adopters is a great way to establish credibility and will serve as a foundation for product expansion and help generate large quantities of validation data that would otherwise be very costly to acquire by the company alone. The flexibility of expanding product offerings is an advantage of the product-based business model; however, focusing on the first minimal viable flagship product is required for success.

Companies such as OrganoBiotech, Aim Biotech, Mimetas and Insphero have adopted a product-based business model to commercialize their platform technology. The business model offers substantial advantages in terms of scalability. By creating a standardized, ready-to-use platform, the company can produce their product in bulk, leading to cost efficiencies and consistent quality. This approach not only simplifies business operations but also expands the reach of the technology, making it accessible to a broader range of institutions and researchers to establish a strong market presence. This business model aligns with the needs of the fast-paced scientific community, providing immediate solutions that accelerate research and development processes.

recreated in vitro through the manipulation of Wnt, BMP and SHH signalling pathways. These region-specific organoids can be studied alone for drug toxicity screening or fused together as assembloids[9] to recreate interneuron migration[19] and neuron projections[111] or to study complex neural circuits and microglia migration after injury[112]. Organoids and assembloids can be used as disease models for genetic structural deformities to recapitulate disease mechanisms[9,101].

Cerebral organoids also suffer from the lack of integrated perfusable vasculature, which ultimately limits their growth and functional maturation. Incorporating endothelial and mural cells enables spontaneous vessel formation[113]. Fusion of vascular organoids with cerebral organoids can recapitulate the neovascular interaction[114].

Brain-on-a-chip devices often focus on recapitulating the highly selective endothelial barrier, the blood–brain barrier (BBB). An on-a-chip conduit is commonly endothelialized with endothelial cells, supported by pericytes and co-cultured with astrocytes and neurons. Through the application of oscillating shear, BBB-on-a-chip achieved over 4,000 Ω cm^2 of transepithelial electrical resistance[115], which is within the in vivo BBB range (1,500–8,000 Ω cm^2)[116]. By precisely controlling the sizes and shapes of the 3D-printed endothelialized channels, physical and molecular mechanisms of cancer extravasation can be investigated in vitro[117]. Nevertheless, recreating the hierarchical branching of vessel networks under 50 μm in diameter remains difficult.

The typical integration of organoids into OoC devices includes the design of a culture chamber for the maintenance of cerebral organoids, whereas the media is perfused through the adjacent conduits[28,118–120] (Table 1). Organoids are commonly transferred into the device after the entire differentiation process[118,119] and encapsulated in hydrogels[28,118–120]. When cultivated for up to 30 days, the cerebral organoids demonstrate ventricle formation[118] and subventricular[28] and cortex[120] zone development with their respective signature gene expressions, enabling modelling of prenatal exposure to nicotine and cannabis[28,118]. When cerebral organoids were used as a supporting material in the bath for 3D printing, a conduit network was 3D printed in them for vascular perfusion[121]. By incorporating a micropillar array, pluripotent or adult stem cell aggregates or organoids can be trapped within a microfluidic chip without the use of hydrogels, thereby improving nutrient and oxygen transfer[122]. Immune cells were also incorporated within the microfluidic chip with brain organoids to mimic injury response[123].

Further studies are needed to incorporate a functional endothelial compartment in integrated approaches because an organoid often forms a necrotic core when the size reaches the millimetre scale[28].

Table 1 | Examples of integrated systems

Tissue	Organoid	Organ-on-a-chip	Advantages of integration	Challenges	Applications	Ref.
Intestine	Patient-derived colorectal organoids, HUVECs and primary lung fibroblasts	IFlowPlate[a]	Intravascular perfusion; intertwining of colon organoids and vasculature	Enclosed and inaccessible luminal space; inability to assess barrier function	Studying immune cell recruitment, attachment and infiltration during inflammation	49
	Patient-derived organoids and gut-specific microvascular endothelial cells	Intestine Chip/Emulate[a]	Luminal flow; mimicking peristaltic motion through cyclic strains	Lack of cellular complexity, such as fibroblasts, immune cells, nerve cells	Studying nutrient digestion, absorption and transportation; evaluating drug delivery, toxicity and efficacy; modelling host–pathogen responses	56
	Patient-derived organoids and human colon epithelial cells	Colon Chip/Emulate[a]	Perfusion and control of flow; non-invasive imaging of mucus layer	Lack of vasculature and peristaltic motions; lack of tubular geometry in epithelial layer	Recapitulating mucus layer, its structure and function	57
	Organoids from human duodenal biopsies	Microfluidic device with patterned middle hydrogel chamber flanked by two perfusable channels	Perfusion; long-term organoid stability	No microvasculature in the parenchymal space	Disease modelling; drug discovery; modelling of parasite infection	59
	Human small intestinal cell organoids obtained from duodenum biopsies	Vascularized intestine on a microfluidic device	Perfusable vasculature; increased organoid stemness and survival	Needs further investigation of the angiocrine mechanism	Studying endothelial-mediated intestinal homeostasis	178
Kidney	hES cell-derived and hiPS cell-derived kidney organoids	3D-printed microfluidic chip	Perfusion; enhanced vascularization and maturation of kidney organoids	Achieving and sustaining over long-term physiological pressure differences; integrating various kidney compartments (for example, glomerulus, proximal tubule) into a functional nephron	Studying kidney development, disease and regeneration	70
Lung	hES cell-derived lung progenitor cells	Micropatterned PDMS arrays	Directed differentiation driven by patterned architecture and tubular structures	No perfusion, no mimicking of breathing motion	Studying differentiation of lung progenitors by geometry-driven mechanism	82
Liver	hiPS cell-derived liver and islet organoids	Multi-organoid-on-chip system	Organ–organ crosstalk under perfused co-culture conditions	Lack of endothelial cells	Liver–pancreas islet insulin and glucose regulation kinetics	86
	iHep and HUVEC	Perfusable microfluidic device	Vascularized organoids	Lack of perfusable vasculature	Multi-organ system to study liver–intestine–stomach axis	87
	hiPS cell-derived liver organoids and $CD8^+$ T cells	Microfluidic chip	Co-culture of liver organoids and T cells	Lack of supporting cellular complexity, such as other immune cells, Kupffer cells and endothelial cells	Studying adaptive immune response to hepatitis C virus	89
	HepaRG progenitor liver organoids	SteatoChip	Mimicking of endothelial cell fenestration through micro-barriers	Lack of endothelial cells	Drug screening for testing for non-alcoholic fatty liver disease; on-chip differentiation	91
	iPS cell-derived liver buds organoids	Omni-well array culture platform	Scalable and reproducible production	Absence of flow in system	Disease modelling and drug screening	92
Pancreas	hiPS cell-derived organoids	Multilayer microfluidic device	Perfusable 3D culture; generation of heterogeneous islet organoids	Human islet maturation and functional monitoring	Diabetes modelling and drug testing	98
	Patient-derived organoids, PDECs and islet cells	Multilayer microfluidic device	Cell–cell interactions in patient-derived cells; crosstalk between PDECs and islet cells	Low viable cellular yield when using human tissues	Cystic fibrosis-related diabetes	97

Table 1 (continued) | Examples of integrated systems

Tissue	Organoid	Organ-on-a-chip	Advantages of integration	Challenges	Applications	Ref.
Brain	hiPS cell-derived brain organoids	PDMS-based device with five parallel functional channels interconnected by micropillar structures	Perfusion with physiological flow rates; high cell viability; heterogeneous regions	Poor organoid maturation; lack of control in conventional culture	Studying prenatal nicotine exposure, on-chip differentiation	118
	Brain organoid differentiation-on-chip	Air–liquid interface created by microfluidic channels	Perfusion enables organoid nourishment, preventing necrotic core formation; uniform size and subventricular zone development	Prenatal maturation in organoids; no endothelial barrier function	Studying prenatal tetrahydrocannabinol and cannabis exposure	28
	hiPS cell-derived brain organoids, on-chip differentiation	3D-printed, perfusable chamber	Perfusable vascular network interacting with the organoids; isogenic cell sources	Lack of off-chip control	Modelling of neurovascular interactions	119
	hES cell-derived brain organoids	Microfluidic system with membrane separating the culture and the perfusion chamber	Enables compression application, planar growth of organoids, convolution of cortex region and high cell density; observation of organoid zone formation	No endothelial barrier function or endothelial–parenchymal interaction crosstalk	Modelling brain organoid development and maturation	120
	hiPS cell-derived brain organoids	SWIFT printed channels within organoid-embedded hydrogel	High cellular density with perfusable channels at different diameters; large tissue construct		Improved tissue viability through perfusion	121
	hiPS cell-derived brain organoids, on-chip differentiation	Microfluidic device with micropillar array	Elimination of Matrigel for organoid entrapment		Elucidating effects of breast cancer-derived exosomes	122
	hiPS cell-derived brain organoids, on-chip differentiation	MEA mesh	In situ differentiation; long-term monitoring	Rigid MEA limits the functional development of organoids	Long-term cultivation, built-in functional readout; non-invasive monitoring of electrophysiology	53
Spine	hES cell-derived organoids	Membrane holder for MEA plug-in	Perfusion control; improved viability and functional maturation	No endothelial barrier function or endothelial–parenchymal crosstalk	Recapitulating the biology and electrophysiology of human nociceptive neurons and dorsal horn interneurons	124
Retina	hiPS cell-derived retinal organoids	Microfluidic channels	Vascular-like perfusion allows constant media supply; co-culture of various retinal cell types	Functional maturation; long-term culture potential	Recapitulating interactions between RPE and retina photoreceptors; studying drug-induced retinopathy	126
Heart	hiPS cell-derived cardiac organoids	Micropatterned arrays	Biophysical microenvironment for stem cell differentiation and cardiac function modulation	Require more physiologically relevant 3D cues	Developmental toxicity testing	141
	hiPS cell-derived cardiac organoids	Micropatterned sheet	Branched vascular networks through micropatterning of hiPS cells	Lack of perfusion	Modelling earliest stages of human cardiac vascularization	142
	hiPS cell-derived cardiac organoids	3D-bioprinted chambered cardiac pump	Perfusion and pump function	Low pump function and ejection fraction	Potential application in health and disease tissue remodelling	147
	hiPS cell-derived cardiac organoids	Micropatterned array	Confined shapes/ microenvironment for differentiation; cardiac chamber formation	Lack of perfusion and chamber filling	Potential application in health and disease tissue remodelling	143

Table 1 (continued) | Examples of integrated systems

Tissue	Organoid	Organ-on-a-chip	Advantages of integration	Challenges	Applications	Ref.
Tumour	ISO-50 human colorectal cancer organoid	Microfluidic device with flow around Matrigel domes of organoids	Substantial increase in organoid formation efficiency	Lack of microvasculature of CAFs, TAMs, TECs and microenvironmental cues (for example, hypoxia, pH gradient); device material (PDMS) drug absorption	Drug testing with flow; organoid expansion	152
	Small cell lung cancer organoid	Microfluidic device with organoid reservoir and a fluid flow channel	Assessment of influence of flow on drug testing in organoids	Lack of vascularization and TME cellular components, such as CAFs and MACs; no chemical gradients; device material (PDMS) drug absorption	Enhanced method for drug testing on organoids	153
	Patient-derived pancreatic cancer organoids	PDMS-based microfluidic device with perfusion	Incorporation of flow within the system		Tumour intravasation; drug testing	155
	Human colon tumour organoid	Microfluidic device with chambers for organoid culture	Assessment of cancer organoid response to shear forces		Effect of peristalsis flow on organoid growth and deformation	154
	PDO	Microfluidic device with middle microvasculature channel flanked by perfusable channels	Assessment of tumour growth and vascularization		Cancer angiogenesis	157
	Pancreatic cancer organoids and colon organoids	PDMS-based microfluidic device with a channel for fluid and drug flow	Assessment of organoid response to flow of different drugs		Real-time monitoring of cell death and growth after exposure to drugs	158
	Patient-derived human mesothelioma organoids	Multilayered microfluidic device with in situ crosslinking of hydrogel-embedded organoids	New ECM that crosslinks in situ via UV light		Drug testing with flow	160
	PDO	TRACER (paper-based scaffold rolled around a central mandrel)	Generation of hypoxia gradients to study tumour growth and cytokine secretion		Hypoxic progression of cancer	193
	PDO	inVADE	Control of flow; co-culture with endothelial cells and fibroblasts	No microvasculature in the parenchymal space	Predicting cancer progression; drug screening	159
Liver, heart	iPS cell-derived liver and cardiac organoids	Multi-organoids-on-a-chip device	Organ–organ crosstalk under perfused co-culture conditions	No endothelial cells; basic characterization of cardiac tissue function	Liver–heart axis to study anti-depressant drug responses	167

CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; hiPS, human induced pluripotent stem; hES, human embryonic stem; HUVECs, human umbilical vein endothelial cells; iHep, induced hepatic cells; iPS, induced pluripotent stem; MACs, macrophages; MEA, microelectrode arrays; PDECs, pancreatic duct epithelial cells; PDMS, polydimethylsiloxane; PDO, patient-derived organoid; RPE; retinal pigment epithelium; SWIFT, sacrificial writing into functional tissue; TAMs, tumour-associated macrophages; TECs, tumour endothelial cells; TME, tumour microenvironment; TRACER, tissue roll for analysis of cellular environment and response; UV, ultraviolet. [a]Commercialized.

Therefore, 3D printing techniques have been used to develop a microfluidic platform with endothelialized perfusable channels and a middle reservoir hosting isogenic cerebral organoids supported by sprouting endothelial cells[119].

OoC devices can also topographically guide organoid growth. For example, confinement between a glass cover-slip and a membrane on top of the perfused channel forces cerebral organoids to grow planarly to facilitate in situ imaging[120]. The human brain is characterized by prominent folds, that is, convolutions. Using blebbistatin and CRISPR–Cas9-edited cells, two opposing forces which were required for these extensive convolutions were identified: cytoskeleton contraction in the organoid core and nuclear expansion at the organoid perimeter[120].

Continuous non-invasive recording of functional electrophysiological readouts, such as extracellular field potentials that constitute standard electroencephalogram recordings, is key to building a versatile organoid-on-a-chip platform to investigate electrical activity in the brain. For example, 3D flexible microelectrode arrays (MEAs) integrated into a microfluidic system for organoid culture would be an ideal next step towards this effort. Brain organoids with a mesh-like MEA were cultured for 40 days, enabling extracellular recording of spontaneous action potentials with a magnitude of 50 μV (ref. 53), compared with typical human EEG signals in the range of 10–100 μV (Table 1).

Spinal cord. The spinal cord transmits motor commands from the brain to the body and transmits the sensory feedback in reverse. A human spinal organoid-on-a-chip device was used for modelling the biology and electrophysiology of human nociceptive neurons and dorsal horn interneurons in nociceptive circuitry, which is essential for the development of new pain therapeutics[124]. This device was constructed by integrating a membrane with a 3D-printed organoid holder to enable the plug-and-play measurement of organoid electrical activity using MEA plates for testing nociceptive modulators, mustard oil and capsaicin; however, these measurements were not compared to human or animal model physiological readouts and may therefore lack

the necessary validation to ensure that the responses are indicative of authentic physiological reactions[124] (Table 1).

Retina. The retina constitutes the light-sensitive layer at the back of the inner eye that converts the light signals into electrical pulses, enabled by precise cellular arrangements of rod-like and cone-like neural receptors. Retinal organoids exhibit native-like polarity, which cannot be observed in animal models[125]. A membrane-containing microfluidic device enabled the co-culture of retinal pigment epithelium and retina organoids while providing perfusion[126]. The epithelium was seeded on top of the membrane, where the retinal organoids were situated with photoreceptor cells protruding out of the organoid surface, allowing the establishment of a defined interaction site between the segmented structures of the retinal organoids and retinal pigment epithelium to model drug-induced retinopathy[126] (Table 1). The system enabled the assessment of vascular endothelial growth factor A secretion and toxicity induced by chloroquine application.

Heart

Heart function relies on cardiomyocyte contraction enabled by the electromechanical coupling and extensive vascular network for nutrient and oxygen supply[127]. Both cardiac organoids and OoCs rely on hES cell and hiPS cell differentiation as adult cardiomyocytes have limited proliferation potential. High-fidelity cardiac organoids enable the study of cell–cell and cell–matrix interactions in heart development[128] and disease modelling[129]. Nonetheless, most cardiac organoids only contain a portion of the heart components, without a complete recapitulation of spatial functional differentiation and a four-chambered structure. Moreover, it remains challenging to create stable and perfusable vascularized cardiac organoids[130,131].

Heart-on-a-chip devices enable measurement of forces in cylindrical myocardial tissues anchored at the two ends while providing maturation-inducing mechanical or electrical stimuli, which are critical for capturing disease phenotypes[35,132–138]. Microfluidic devices with pump and plate-like OoCs provided flow and vascularization around these bundles[139,140]. OoC approaches could therefore facilitate the integration of electrical signals, mechanical stretching, ECM cues and perfusable vasculature into the organoids.

Micropatterning techniques offer geometric confinement to improve structural morphology and contractile function of cardiac organoids[141], enabling the scalable formation of an organized germ layer and promoting the formation of vascular networks[142,143] (Table 1). Organoids can improve cardiac maturation as demonstrated in multilineage organoids undergoing a sequence of morphogenic events to co-develop gut and heart tissue[144].

Despite substantial progress in the development of human chambered organoids[145,146], robustly recapitulating cardiac chambers is still a challenge for both fields, which may be overcome by integration. For example, electromechanically functional and chambered cardiac ventricles bioprinted with mixtures of hiPS cells and ECM facilitate in situ cardiomyocyte differentiation to yield an ejection fraction (that is, the percentage of the liquid pumped out of the ventricle at each beat) of 0.7% (ref. 147) (Table 1). Integrated systems will advance studies of cardiac genetic disease while providing in situ functional readouts and the cellular complexity necessary to accurately capture complex phenotypes.

Tumour

Traditionally, patient-derived tumour cells are transplanted into mice to form patient-derived xenografts, with the disadvantage of mouse-specific tumour evolution[148]. Cancer cells can also form patient-derived organoids in vitro. Multipotent cancer cells exhibit chemoresistance and differentiate to capture tumour heterogeneity[149]. Organoids are most often cultured and expanded in Matrigel domes lacking many inherent complexities of the tumour microenvironment, such as shear forces, 3D orientation, mechanical cues, environmental gradients (such as oxygen, pH and cellular polarization), vascularization and supporting cells[150]. Inclusion of the organoids into OoCs can better mimic tumour invasion, extravasation and angiogenesis, as well as interactions with other cells, such as cancer-associated fibroblasts, tumour-associated macrophages and tumour endothelial cells[151]. Flow around tumour organoids enhances their growth rate[152], increases throughput of monodisperse organoid culturing[153] and enables mimicking of peristaltic motion, such as that in human colon carcinoma[154]. Compartmentalization of flow versus organoid channels further facilitates the studies of cell migration and stromal contributions[155].

An important hallmark of cancer is the secretion of cytokines and chemokines leading to the ‘leaky vasculature’, the main target of monoclonal antibody-based treatments. OoC approaches are well suited to capture endothelial cell migration and branching towards the organoid[156], to provide physiological convection–diffusion mass transport via engineered vasculature[157] and to facilitate drug testing[158] (Table 1). For example, pancreatic cancer organoids have been incorporated within the inVADE (integrated vasculature for assessing dynamic events) platform, a 96-well based perfusable plate with an integrated polymeric blood vessel connecting a unit of 3 wells, to study fibroblast matrix deposition and inhibition of drug transport[159] (Fig. 2d). Further challenges for integrative approaches include improving microvasculature in the parenchymal space and incorporation of tumour-associated macrophages and T cells.

Standard cancer organoid formation relies on temperature-sensitive hydrogels, which limit their application into enclosed microfluidic channels. Alternative materials, such as photocrosslinkable hyaluronic acid-based hydrogels, can be used to enable complex seeding in enclosed OoC devices[160]. Tumour organoids also require benchmarking validation to achieve higher clinical relevance as the high degree of biological variability resulting from patient-derived cells can lead to morphological and phenotypic inconsistencies[161]. Moreover, genomic instability of cancerous cells might give rise to variable organoid properties[162]. In vitro validation of physiological relevance is particularly challenging for tumour models due to the high variability of human phenotypes, even for the same tumour type, and tissue heterogeneity, impacting both organoids and OoCs. Owing to their defined geometries, these measurements can be relatively easily implemented in OoC devices and screened in a high-throughput manner.

Multi-organ systems

Multi-organ systems are formed using assembloids and body-on-a-chip approaches. For example, cortico-striatal assembloids were used to study the effects of chromosome 22q13.3 deletion on calcium activity[163]. More recently, attempts have been made to incorporate brain-spinal cord assembloids within microfluidic setups to monitor neural signal transmission in situ[164].

Assembloids are suitable when modelling tissues that reside in close proximity to one another in the body; for example, a bladder assembloid containing an outer muscle layer and inner epithelial layer resulted in the enhancement of tissue maturation[165]. By contrast, OoCs

are more useful when modelling the interaction of distant organs. For example, a pump-free multi-OoC system was used to predict drug efficacy and metabolic conversion in the liver, tumour and heart systems while recirculating serum-free medium between the compartments[166]. In a two-chambered organoids-on-a-chip device, the hepatic enzymes released by the liver organoids metabolized the antidepressive drug clomipramine into an active metabolite that impacted cardiac organoid function, indicated by reduced beating and calcium flux[167]. Yet modelling true organ–organ interactions requires functional vasculature for communication. This is one of the most challenging tasks for OoCs due to the complexity of fluid handling and scaling laws[168] that govern relative ratios of different organ compartments and complicate translation (Box 2).

Moreover, a substantial hurdle in multi-organ systems lies in the disparity among various differentiation media required for different organoids and tissues. To overcome this limitation, matured human heart, liver, bone and skin compartments were cultured in organ-specific culture media and interconnected through a vascular system composed of a membrane coated with endothelial cells that served as a selective barrier for each compartment[169]. All tissues went through the state-of-the-art organ-specific maturation protocols and passed their respective functional assessments, for example, contractility assay for cardiac tissues, bone density quantification, barrier function of skin tissues and albumin secretion of liver tissues[169]. These tissues and the corresponding endothelium maintained their organ-specific phenotype after 4 weeks of organ–organ crosstalk, demonstrating the pharmacokinetics, pharmacodynamics and cardiotoxicity of doxorubicin. The mixed model, without the endothelial barrier and individual tissue chips, did not present equivalent results, indicating the importance of a functional endothelium[169].

Although multi-organ systems are promising, the preparation of organ-specific organoids, expertise in microfabrication, and the associated costs and labour can substantially limit their adoption. The difficulty of recapitulating the physiologically relevant cell-to-media ratios is another obstacle in the field.

Vascularization

Vascularization is a grand challenge for both organoids and OoCs, as well as for regenerative medicine and tissue engineering (Fig. 1). The diffusion limit for oxygen in tissues of physiological cell density is thought to be on the order of ~100 µm (ref. 170) – values higher than this will cause insufficient nutrient and oxygen supply to the inner core, leading to necrosis[130]. Vascularizing organoids is important for paracrine communication, the application of mechanical stimulation, tissue maturation, endothelial barrier function and communication in multi-organoid systems. Although organoids have been wrapped in self-assembled perfusable vasculature[49] and vascularized in vivo[171,172], thus far, there has been no evidence of a successful and addressable vasculature within organoids in vitro[173].

Blood vessels can be bioengineered in vitro following two general approaches: the first is the self-assembly method, where endothelial and supporting cells are mixed within hydrogels to self-assemble into lumen-containing vascular networks (such as in iFlow plates[49]; Fig. 2b). The culture substrate topography (for example, a surface with groves and ridges) and the release of angiogenic factors can induce directional self-assembly[174]. The supporting cells are crucial for this process as they secrete essential cytokines, such as angiopoietin 1 and hepatocyte growth factor[130]. The presence of physiological flow further enhances barrier function[175]. Although the self-assembled vessel diameters are on the order of those of capillaries, long-term perfusion may be challenging and the incorporation of functional cell types (such as cardiomyocytes) may disrupt network stability.

The second approach consists of fabricating hollow channels and networks within hydrogels or polymeric materials[176], followed by endothelial cell seeding, resulting in vascular networks. The main disadvantage is diameter control, usually in the range of the diameter of venules and higher (>100 µm). This approach enabled the development of a thrombosis model, a chip that consists of an endothelium perfused with human whole blood[177].

Microfluidic devices can help control the flow in vascularized kidney[70] (Fig. 2c) and small intestinal cell organoids[178]. Moreover, the self-assembly method for vasculature formation can be combined with pre-established polymer conduits lined with endothelial cells to achieve hierarchical branching[179].

Despite the creation of vascular lumens in natural hydrogels being easy to achieve, their weak mechanical properties cause them to collapse as the cells remodel the matrix. Although synthetic polymers are strong and can be processed into lumens by additive manufacturing, their permeability to proteins and cells is lacking. To solve these issues, a branching AngioChip[176] was developed by 3D stamping of poly(octamethylene maleate (anhydride) citrate) polymer with micrometre-sized holes in the vessel wall (Fig. 2e), which enabled communication between the vascular and parenchymal compartments. By scaling down AngioChip (requiring 2 million cells per tissue) to a single vessel, stem cell-derived vascularized tissues were cultured in the inVADE platform incorporating an endothelialized poly(octamethylene maleate (anhydride) citrate) vessel (Fig. 2d), the size of a venule, spanning a column in a 96-well plate. This strategy enabled cultivation of both single and duo-organs connected by vasculature, which required only 200,000 cells per tissue[139]. Other biofabrication approaches have demonstrated vascular lumens with cell infiltration[173,174,178], including those from commercial sources, such as Nortis, Emulate, Aracari, AIM Biotech or Mimetas[8,180].

One of the main obstacles in the vascularization of pluripotent or adult stem cell-derived organoids is the mismatch between the differentiation medium of the stem cells and the pro-angiogenic medium to support vessel formation. Moreover, the presence of endothelial cells and the paracrine cytokines they secrete can disrupt differentiation. Often, organoids are differentiated separately and then seeded on top of a vascular bed. Alternatively, differentiated organoids can be combined with isolated endothelial and supporting cells (such as mesenchymal stem cells)[92] to induce vascularization. Another possible mismatch arises in the context of hydrogels used to embed tissue-specific organoids, which must be compatible with a hydrogel that can promote vessel network formation, requiring substantial reduction or removal of Matrigel[49].

Outlook

The level of challenge in implementing an integrated approach depends on the existing expertise of researchers. Researchers skilled in organoids can start with off-the-shelf[8], open (as in well plate-like versus closed microfluidic chip-like) OoC systems, whereas those skilled in OoCs can begin by using commercially available organoid differentiation kits to reduce variability. For beginners, it is recommended to first gain experience with commercially available systems for each method separately before attempting to integrate them. It might be better to start with organoids as OoC systems would require some

hardware or clean room microfabrication facilities to customize the integrated platforms.

Integration approaches require the development of designer biomaterials to enable control of the shape and size of organoids to drive the deterministic tissue patterning in a hydrogel[58,59]. Reliance on thermogelling hydrogels, such as Matrigel, that suffer from batch-to-batch variability is a key limitation. Determining at what stage of differentiation organoids should be placed into an OoC device is another challenge. Terminally differentiated cells proliferate less and are more fragile. If progenitors are used, on-chip differentiation is required, complicating culture media and matrix selection, especially in multi-organ systems.

Applying physical stimulation (electrical, mechanical, flow, and nanometre to micrometre topography) is a key advantage of the OoC approach[169]. Most often, these cues are applied in a metronomic fashion, unlike chaotic and fractal cues that underpin physiological systems[181]. Changes in pressure across various barriers, as they occur in the body, are largely missing. These features often drive physiological maturation, for example, in glomerular slits[182]. Introducing such physiological complexity into stimulation patterns may further improve the fidelity of both OoC and organoid systems.

Vascularization has been a challenge for all fields. Creating a stable, addressable and perfusable vasculature that lasts for months, in and around organoids, is the holy grail of the integration approach. It remains to be determined if organ-specific endothelial cells are required. Flow throughout OoC devices could be used to stimulate the differentiation and stabilization of organ-specific vasculature within the organoids themselves. Additional cells (such as resident macrophages) and biologics (such as exosomes) could stabilize the vasculature across the entire parenchymal tissue.

Another challenge is connecting different inputs and outputs to the right cell type in the integrated system to create a truly functional system. For example, kidney organoids contain proximal tubule cells, glomerular podocytes and endothelial cells. In vivo, the flow at the two sides of these epithelial and endothelial barriers is highly compartmentalized. Configurations are just emerging that connect the two units, glomerulus and renal proximal tubule, in a single system in series with flow moving from the glomerulus to the proximal tubule unit as in the body[183].

The more complex the model, the harder its adoption into industrial practices and workflows. Organoids, OoCs and their integrated platforms will need to be consolidated into an automated cell culture and characterization workflow. Automation can substantially improve the consistency, reproducibility and efficiency of 3D cultures, reducing human error and accelerating the lead time for drug candidates. Scalable and automated production of OoC and integrated devices will ensure the field moves beyond traditional screening in a handful of well plates. This necessitates processing of plastics via hot embossing or injection moulding and moving away from standard soft lithography and PDMS. As OoC approaches become mainstream, the amount of plastic waste from these devices will increase. This will require the transition to processing of plastics that are biodegradable on demand but still maintain optical clarity and possess thermoplastic properties for scalable production. Ideally, such polymers will be obtained from monomers that are available entirely from renewable sources (for example, through fermentation).

3D bioprinting is an effective way to fabricate OoCs and sophisticated assembloids[184], including both fabrication of the platform itself and cell deposition into the platform. Multi-material processing is required to place hard and soft polymers, conductive materials,

Box 3 | Validation of integrated organoid and organ-on-a-chip models

There is a need for common and universally accepted criteria and hallmarks to validate integrated organoid and organ-on-a-chip models[1]. This includes both validation of the device microenvironment and the resulting phenotype of the 3D tissues. Regarding device operation, the following additional points need to be considered: first, media-to-cell ratios that recapitulate native concentrations of secreted factors. This aspect is rather difficult to model and benchmark due to fabrication constraints, requiring 10–100 µm-scale wells for cell cultivation, yet hundreds of microlitres to 1 ml volumes of media to enable efficient perfusion, all of which increase the cell-to-media ratio above the physiological limit. Second, assessing functionality of the nascent vasculature: characterization of dimensions and permeability to small (~100 Da) and large (~10–100 kDa) molecules should be performed to benchmark against known values for human vasculature. Third, recapitulating changes in pressure across various barriers in the human body. These parameters are known but are difficult to model. They often drive physiological changes, such as the maturation of glomerular slits. Thus far, most 3D models have been mechanically too fragile to mimic these features. Measuring and reporting the resulting pressure differences would be important.

Validation of integrated tissue phenotype should include the comparison of RNA and protein expression signatures to native human organ signatures or those of explanted tumours, along with immunostaining comparison of the integrated models to those of native tissues. This is particularly important for highly heterogeneous tumour tissues. The wealth of single-cell sequencing data from the Human Cell Atlas could facilitate this task. Additional data with clinical parameters can be obtained from the United Network for Organ Sharing and used to train machine learning algorithms to classify the integrated system with respect to native human organs. Moreover, we propose quantifying cytokine secretion and functional comparisons to further prove biological relevance, such as characterization of mechanical properties (like tumours being generally stiffer than native tissues), characterization of permeability, impulse propagation and contractile force as appropriate and determining half maximal effective concentration (EC_{50}) for toxicity of known drugs. Liver and heart tissue, being the primary locations for drug toxicity after approval, have been the focus of in-depth in vitro modelling. To validate these new models, a collaborative initiative has been formed among academic institutions, industry and regulatory bodies. This collaboration is manifested through the IQ Consortium, which focuses on liver models, and the Comprehensive In Vitro Pro-arrhythmia Assay (CIPA) initiative, dedicated to cardiac models. These initiatives provide guidance on the panels of known drugs and physiological indicators useful for model validation. Ideally, criteria for other models will be further defined through such collaborative efforts.

and hydrogels at the right locations of the OoC device to achieve physiological cell assembly and sensor incorporation. Bioprinting enables precise positioning of different cell types as well as organoids in OoC devices.

As production throughput increases, handling flow becomes an issue. Microfluidic setups with open well configuration will therefore have the potential for greater scalability because they do not require additional connection of tubing and pumps. The unphysiologically high media-to-cell ratio is a challenge and may result in dilution of signalling molecules, requiring iterative optimization of microfluidic design.

The ability to extract data at the cellular level in an in vivo-like human cell environment is a fundamental advantage of OoCs and organoids compared with animal models. Current efforts should focus on extracting information from the model, that is, scalable analytics, in addition to building better models. Up-to-date, functional readouts in most OoC devices rely on semi-manual approaches, where either image analysis or sample analysis happens off-chip[35]. This means that sampling media to read the concentrations offline or tracking the movement in the device via microscopy and image analysis will have to be replaced or upgraded to render them compatible with high-throughput screening setups, routinely used in the pharmaceutical industry to enable screening on the order of 10,000 compounds over a period of 2–3 days. New sensor technologies can now be integrated into OoC devices[50,51,185,186], and tissue clearing techniques and light-sheet microscopy can be used to image complex integrated models in 3D. Moreover, incorporating computer vision and deep learning approaches will enable collection and analysis of readouts while simultaneously finding emerging data trends from such highly scaled and integrated approaches.

The issues of cell line and batch-to-batch variability that are often reported for the cultivation of primary cells and the differentiation of pluripotent or adult stem cells need to be overcome. Directed differentiation protocols have gone a long way since the generation of iPS cells in 2006 (ref. 187). The use of defined media and small molecules instead of growth factors is now common[188]. An important limitation in the integrated approaches is that certain cell types might secrete factors that may lead to death or a functional deficit of other cells, such as skeletal muscle and adipose tissue wasting and cardiac dysfunction in cancer[189]. Highly specialized supplements of current culture media that are generally designed to enable a single cell type to thrive require the issue of common culture media to be addressed. Further understanding of the impact of circadian rhythm may be necessary to reduce variability.

Reporting the sex of the cells and designing experiments with models with different sex characteristics will enable capturing of sex-specific drug responses and disease manifestations. This process can be accelerated if all cells in a model are derived from isogenic sources, a clear advantage of organoids compared with the combination of already differentiated or primary cells in OoCs. Importantly, with the OoC approach, both the influence of sex as a genetic variable and the presence of sex hormones can be investigated to develop the much-needed models of female-to-male or male-to-female transition and better understand physiological complexities of a continuum of hormone concentrations, precisely controlled by flow, that may underpin diversity.

Finally, it is necessary to define common and universally accepted criteria and hallmarks for integrated model validation and benchmarking compared with in vivo organs (Box 3). Because they are the most common sites of post-approval drug toxicity, liver and heart tissue have been extensively modelled in vitro. Working together to validate recently developed models, academia, industry and the regulators have joined forces through the IQ Consortium for liver models[190] and Comprehensive In Vitro Pro-arrhythmia Assay (CIPA) initiative for cardiac models[191] to develop best practices and define known drug panels and physiological readouts for validation.

To summarize, the rapidly developing fields of organoids and OoCs both aim to develop more relevant human models for drug development and disease modelling. Combining the two approaches in the intestine[59], tumour[159] and kidney[70], enabled capturing phenomena that single approaches cannot; for example, inhibition of drug transport in vascularized tumour organoids[159]. The benefits of integration include the organ-specific cellular hierarchy and structural fidelity inherited from organoids; microscopic features from OoCs guiding tissue morphological formation; better reproducibility and scaling up capacities adapted through OoCs; biocompatible built-in sensors for in situ functional readouts from OoCs; and industrially compatible culture format from organoid cultures.

The inherent variability in stem cell-derived lines and primary cells, as well as the vascularization issues, need to be overcome, especially in using organoid-derived vasculature to drive intra-organoid flow. Scaling device production and developing appropriate artificial intelligence or deep learning approaches to analyse the multitudes of data are required. Validation criteria are needed to evaluate which integrated systems are appropriate mimics of the in vivo environment. This will enable broader adoption in a changing regulatory environment[192]. Unifying integrated systems with artificial intelligence-enabled discovery, which has molecules but no appropriate biology to screen them, promises transformative results.

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Author contributions

Conceptualization: M.R., Y.Z., S.L. and S.O. Writing – original draft: Y.Z., S.L., S.O., C.L., R.X.Z.L., B.F.L.L., Q.W., J.K., K.C., S.R., K.J., B.Z. and M.R. Writing – review and editing: M.R., Y.Z., S.L. and S.O. Visualization: Y.Z., S.L., S.O. and K.C. Supervision: M.R. and B.Z. Project administration: M.R. Funding acquisition: M.R. and B.Z.

Competing interests

M.R., Y.Z. and B.Z. are inventors on an issued US patent for Biowire technology that is licensed to Valo Health; they receive royalties for this invention. B.Z. and S.R. are co-founders and hold equity in OrganoBiotech. The remaining authors declare no competing interests.

Additional information

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