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Current Opinion in Genetics & Development

Modeling development using microfluidics: bridging gaps to foster fundamental and translational research

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In vitro stem cell-derived embryo and organ models, termed embryoids and organoids, respectively, provide promising experimental tools to study physiological and pathological processes in mammalian development and organ formation. Most of current embryoid and organoid systems are developed using conventional three-dimensional cultures that lack controls of spatiotemporal extracellular signals. Microfluidics, an established technology for quantitative controls and quantifications of dynamic chemical and physical environments, has recently been utilized for developing next-generation embryoids and organoids in a controllable and reproducible manner. In this review, we summarize recent progress in constructing microfluidics-based embryoids and organoids. Development of these models demonstrates the successful applications of microfluidics in establishing morphogen gradients, accelerating medium transport, exerting mechanical forces, facilitating tissue coculture studies, and improving assay throughput, thus supporting using microfluidics for building next-generation embryoids and organoids for fundamental and translational research.

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Current Opinion in Genetics & Development 2023, **82**:102097

This review comes from a themed issue on **Early embryonic development models**

Edited by **Miguel Esteban** and **Jose Polo**

Available online xxxx

https://doi.org/10.1016/j.gde.2023.102097

Introduction

Stem cell-based, *in vitro* models of mammalian developments and organ formation are becoming indispensable tools for advancing mammalian developmental biology and disease modeling [1–6]. This is particularly true for understanding human development, given our limited access to and bioethical constraints in human embryonic tissues. Till now, there are various models of mammalian embryo and organ developments, termed embryoids and organoids, respectively, that have been reported [2,3,5,7–9]. Embryoids have been developed to recapitulate early embryogenic events, from pre-implantation blastocyst formation, to peri-implantation and peri-gastrulation development, all the way up to early organogenesis [1–6]. For organoids, there are numerous organoids available now to model the development, homeostasis, and pathology of organs associated with the three definitive germ layers [7–9]. Researchers continuously develop improved embryoids and organoids with enhanced maturity, functions, complexity, structural fidelity, and disease or developmental relevance.

Bioengineering technologies have been used successfully in the development of embryoids and organoids [1,2,4–6,10–21]. These technologies include genetic engineering tools [5,6,10–13], functional biomaterials [14–16,19,20], and bioengineering tools [1,2,5,6,10,11,21] that can efficiently modulate spatiotemporal local tissue microenvironment. Genetic engineering tools are utilized to generate signaling and lineage reporter lines, allowing monitoring of intracellular signaling dynamics and cell fate decisions during embryoid and organoid developments [4,10,12,13]. Genetic technologies have also been utilized to direct cells to interact efficiently with specific chemical cues [5,6,10] or local light illuminations [11,12]. Functional biomaterials, such as synthetic hydrogels [14,15,19,20] and natural extracellular matrix (ECM) proteins [16], have also been used for embryoid and organoid developments, either directly in conventional three-dimensional (3D) tissue cultures [14,15] or in bioprinting [17,18] and microfluidics [19,20]. There are other bioengineering tools utilized to control the size and shape of initial cell clusters for embryoid and organoid developments, such as micropatterning [11,21], AggreWell [2,5,6,10], and microwells [1]. For prolonged embryoid and organoid cultures, tissue culture shakers [6,22] and *ex utero* culture instruments [5,6] have been utilized.

In this review, we focus on discussing promising applications of microfluidics in embryoid and organoid developments. Microfluidic devices can generate gradients of chemical signals, useful for tissue patterning and

symmetry breaking. Through precise controls of microfluidic environments, physical signals, such as gas composition, pressure, and shear stress, can be modulated for embryoid and organoid developments. Since microfluidic devices contain prescribed chambers and channels, useful for loading and positioning different types of cells, microfluidic devices are useful for controlling and studying cell–cell interactions during embryoid and organoid developments. There are also important efforts in developing automated, high-throughput microfluidic devices for embryoid and organoid developments, promising for translational screening applications.

Microfluidic gradients inducing tissue patterning and symmetry breaking

During development, tissue patterning is achieved through specification and differentiation of embryonic progenitor cells into functional tissue cell types in a well-orchestrated manner. The importance of chemical signals, including morphogens, has been well-established in tissue patterning. Morphogen gradients in the extracellular space provide positional information, to which embryonic progenitor cells respond in a dose-dependent manner. Microfluidics offers a convenient platform to create and control graded chemical environments to induce tissue patterning in embryoids and organoids.

Passive diffusion remains the most straightforward way for generating microfluidic gradients. Oftentime, cells are cultured in a microfluidic chamber connected to source and sink reservoirs, which establishes a concentration gradient in the cell chamber following the classic source–sink model of Fickian diffusion. Hydrogels are often added into the cell chamber or between the cell chamber and source and sink reservoirs to prevent advection flows that might cause undesirable effects on cells. Using microfluidic chemical gradients generated using passive diffusion, a broad concentration range of different chemicals have been screened for inducing motor neuron differentiation from mouse embryonic stem cells (Figure 1a) [23]. Microfluidic chemical gradients based on passive diffusion have also been integrated with a 2D micropatterned human gastrulation model to achieve *in vivo*-like axial germ layer patterning, highlighting the importance of combining exogenous bioengineering controls and intrinsic stem cell self-organization to build embryoids and organoids with heightened complexity and *in vivo* relevance [21].

Microfluidic gradients can also be generated through a series of splitting and mixing of microfluidic flows (Figure 1b). Such microfluidic gradient design has been utilized to establish an exogenous Wingless and Int-1 signal gradient to recapitulate rostral–caudal patterning of the neural tube [24]. Interestingly, an isthmic organizer-like region emerges in the patterned neural tube-like structure at the boundary of putative forebrain and midbrain regions, highlighting the autonomy and modularity during organ development.

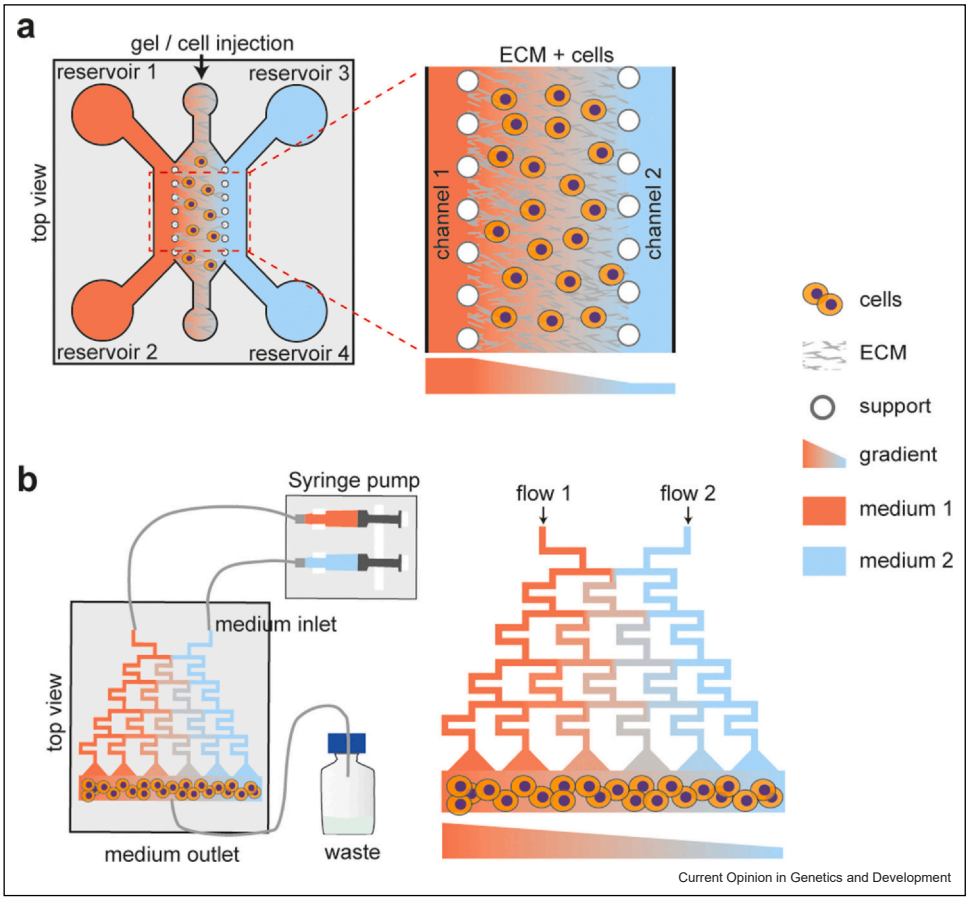
Owing to precisely controlled microfluidic environments, embryoids and organoids developed using microfluidics often show improved efficiency and reproducibility. This feature could be best illustrated using the microfluidic post-implantation amniotic sac embryoid (PASE). The PASE was first developed using a conventional 3D culture, in which a small percentage (5–10%) of human pluripotent stem cell (hPSC) clusters would undergo lumenogenesis, then symmetry breaking, and amniotic patterning, leading to the formation of asymmetric amniotic ectoderm–epiblast pattern that resembles the human amniotic sac [25]. To improve PASE formation efficiency, a microfluidic platform was developed to guide formation of hPSC clusters in prescribed locations before asymmetric morphogen stimulations to drive synchronized PASE formation in a controllable and reproducible manner [3,26].

Microfluidics for controlling material transport and physical environment

Besides chemical signals, other factors, such as nutrients, gases, mechanical forces, and geometric topology, also can have an impact on embryoid and organoid development. Controlled flows in microfluidic devices can enhance nutrient and oxygen transport [27], beneficial to tissue growth, survival, and maturation [16,27–29]. For example, apoptosis was minimized and proliferation was promoted in microfluidic brain organoid cultures (Figure 2a) [16]. Improved survival and insulin secretion were shown in islet organoids under continuous microfluidic perfusion [27,29]. Microfluidics could also influence embryoid and organoid development by removing secreted factors. For example, in a gut organoid chip with independent controls of fluid flow and mechanical deformation, basal flow in gut organoids was shown to induce villi-like morphogenesis of intestinal epithelium, mainly via removal of Wingless and Int-1 antagonists secreted by the tissues themselves [8,30].

Microfluidics has also been utilized for controlling shear stress and hydrodynamic pressure to promote morphogenesis and maturation during embryoid and organoid developments. It has been shown that kidney organoids exposed to high-shear flow exhibited enhanced vascularization and had more mature podocytes and tubular compartments compared with those under static culture (Figure 2b) [31]. Using microfluidics containing a pressure channel, cyclic pressures were applied on colon tumor organoids to mimic peristalsis (Figure 2c) [32]. Applying hydrostatic pressures to mimic transmural pressures on lung explants, transmural pressure was shown to modulate airway-branching morphogenesis,

Figure 1



Morphogen gradient generation by microfluidics. **(a)** Morphogen gradient generated by diffusion through ECM with cells embedded in ECM [23], with (Left) top view and (Right) zoom-in top view. Culture medium is added into 4 reservoirs. Gel and cells are injected into the middle channel. **(b)** Gradient generation by splitting and mixing microfluidic flow [24], with (Left) top view and (Right) zoom-in top view. The flow is driven by syringe pump, and the medium flowing through the microfluidic chip is collected in a waste bottle. Flow 1 and flow 2 in microfluidics systems have different chemical concentrations, which allows for the creation of a concentration gradient within the device.

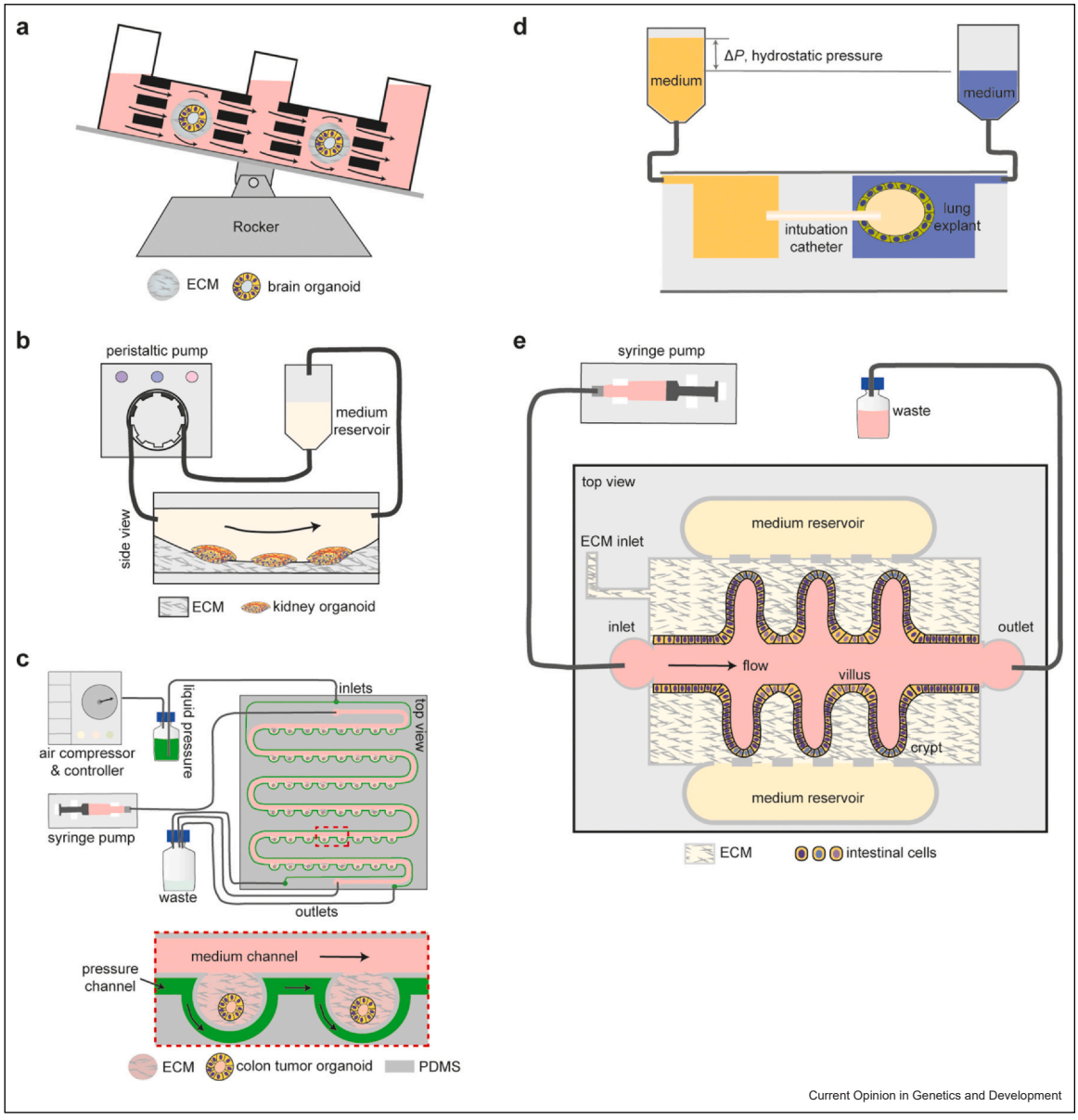
airway smooth muscle contraction, and maturation of lung tissues (Figure 2d) [33].

Microfluidic devices can also provide precise topologies useful for guiding tissue morphogenesis and differentiation. Laser micromachining was applied to fabricate a microfluidic channel in hydrogels for the development of a gut model suitable for long-term homeostatic culture under an external perfusion pump [34]. Topological features of the microfluidic mini-gut model guided the development of intestinal epithelial tissues, leading to the formation of a tube-shaped structure with crypt- and villus-like domains (Figure 2e). Importantly, intestinal stem cells and Paneth cells were exclusively found in crypt-like regions, whereas enterocytes, enteroendocrine cells, and goblet cells were exclusively located in villus-like regions (Figure 2e), mimicking spatial cell organizations in intestinal epithelial tissues.

Microfluidics for controlling tissue–tissue interactions

Tissue–tissue interactions are manifested in every step of mammalian development and organ formation. Microfluidics provides a convenient platform for positioning different tissue cell types at prescribed locations inside a controlled microfluidic environment, imitating *in vivo*-relevant tissue–tissue interactions. To model invasion of extravillous trophoblasts (EVTs) into maternal uterus during the placentation, a maternal–fetal interface was established by seeding EVTs and endothelium cells into two parallel microfluidic channels separated by ECM or a pillar barrier array [35,36] (Figure 3a). The barrier function of the placenta was also imitated by placing trophoblast cells or embryoid bodies in one microfluidic channel to model the embryonic compartment of the fetal–maternal interface and endothelial cells in a separate adjacent channel to model the maternal

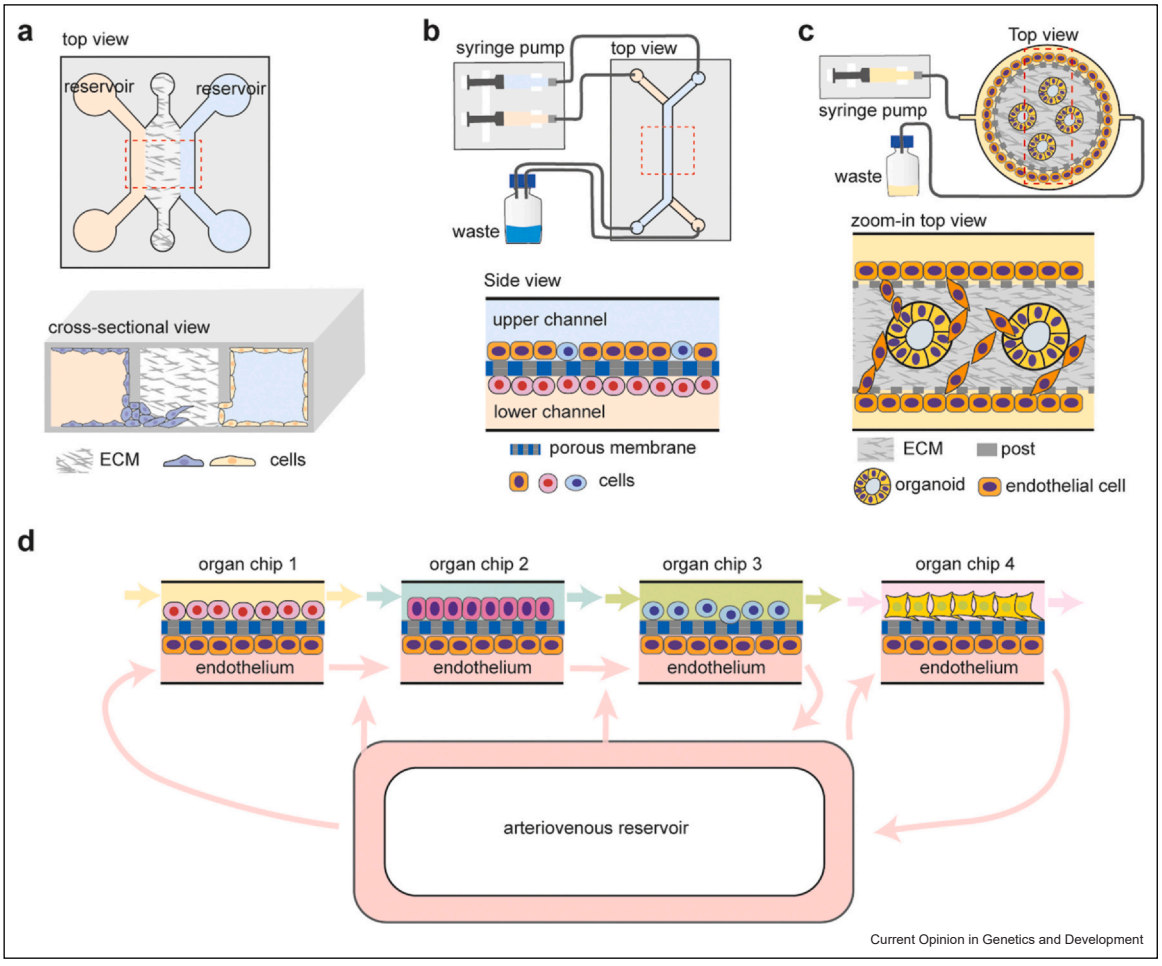
Figure 2



Microfluidics controls material transport and physical environment. **(a)** Microfluidic flow accelerates material transport for brain organoids [16]. The flow in the microfluidic system is driven by a rocker machine. **(b)** Kidney organoids cultured under microfluidic shear flows [31], which is driven by peristaltic pump. **(c)** Colon tumor organoids embedded in ECM experiencing cyclic pressures through the application of a microfluidic pressure channel [32], with (Upper) overall view and (Lower) zoom-in top view. The pressure in the system is regulated through the pressure channel, where the liquid is subjected to increased pressure using an air compressor and controller. The flow within the medium channel is propelled by a syringe pump. **(d)** Lung tissue experiencing transmural pressure difference established using microfluidics [33]. The pressure difference (Δp) within the microfluidic system is established by the difference in heights of the culture medium. **(e)** Microchannel scaffold to guide intestinal epithelial organization and differentiation [34]. In the microfluidic system used for intestinal studies, two independent flows are employed. One flow is responsible for delivering a medium supplemented with nutrients by passive diffusion, while the other flow driven by syringe pump is used for perfusion within the intestinal lumen.

compartment [37–39]. Similarly, by placing different tissue cell types into opposing microfluidic channels (Figure 3b), intra-organ models were constructed, such as a liver model with hepatocytes interfaced with liver sinusoidal endothelial cells, Kupffer cells and hepatic stellate cells [40], and a pancreas model with pancreatic ductal epithelial cells interfaced with islet cells [41]. Vascular and immune systems have also been incorporated into microfluidic organoid cultures (Figure 3c), such as cerebral [42] and hepatic organoids [43,44].

Figure 3



Microfluidics for tissue coculture studies. **(a)** Cells are seeded into two parallel channels separated by an ECM barrier to study cell migration and invasion [35], shown in (Upper) top view and (Lower) side view. **(b)** Cells are seeded at the opposite sides of a porous membrane to study cell–cell interaction by soluble molecules [40,41], shown in (Upper) top overall view and (Lower) zoom-in side view. **(c)** Endothelial cells invading into a center channel containing ECM and organoids for vascularization of organoids [42,43], shown in (Upper) top overall view and (Lower) zoom-in top view. **(d)** Microphysiological systems constructed with multiple organ models on the same chip. Interconnections between organ models are established through arteriovenous reservoir [47,49].

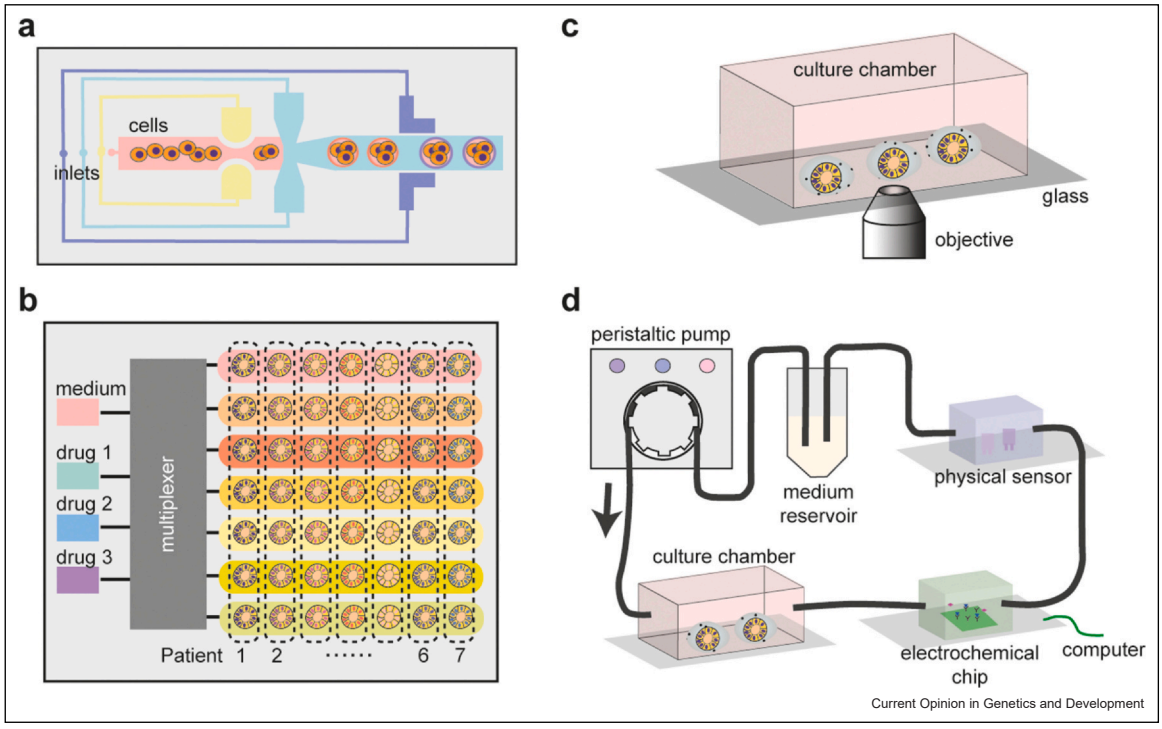
Microphysiological systems containing multiple organ models have been established using microfluidics to study inter-organ communications and model multi-organ processes and systematic diseases [45–49]. Each organ model in the microphysiological system can be maintained in its own optimal condition, and interconnections between organ models are established based on their *in vivo* relationships [47–49] (Figure 3d).

Microfluidics for scalable translational applications

Microfluidics is intrinsically a scalable technology compatible with translational screens. As a potent high-throughput technology, droplet microfluidics, for example, has been used to generate embryoids and organoids with simplified procedures, great throughput, and low variability. So far, droplet microfluidics has been used for the developments of epiblast spheroids [50], liver organoids [51,52], lung organoids [51,53], kidney organoids [51], islet organoids [19,20], mesenchymal bodies [54], and tumor organoids [51,53,55] (Figure 4a). Some droplet microfluidics-based organoid tools have been utilized for large-scale drug screens [51,55]. In another example, an automated microfluidic culture was developed for pancreatic tumor organoids. This system was applied to test up to 20 regimens and 10 patient samples in parallel, offering a promising platform for individual, combinatorial, and sequential drug screens on pancreatic tumor organoids [56] (Figure 4b).

Live imaging is commonly used for analyzing microfluidic organoid and embryoid cultures, given the

Figure 4



Microfluidics for scalable productions of embryoids and organoids. **(a)** Droplet microfluidics for embryoid and organoid generations [19,20]. **(b)** An automated microfluidic system for patient sample screening [56]. The multiplexer is utilized to generate the medium containing various drugs and concentrations. **(c)** Microfluidic chip incorporated with other equipment to be compatible with imaging. **(d)** The microfluidic chip features an integrated sample culture chamber coupled with electrochemical biosensors and physical sensors for real-time monitoring [58]. The flow within the chip is driven by a peristaltic pump.

controlled positioning and orientations of organoids and embryoids in microfluidic devices [3,21,34,56,57] (Figure 4c). *In situ* biochemical sensors can also be integrated with microfluidics, to continually monitor relevant culture signals in microfluidic organoid and embryoid cultures [58] (Figure 4d). These sensors include those for monitoring extracellular microenvironment parameters such as pH, oxygen level, and temperature. Additionally, electrochemical sensors can be utilized to measure soluble protein biomarkers in microfluidic organoid and embryoid cultures. Thus, integration of biosensing technologies with microfluidic organoid and embryoid cultures offers enhanced capabilities for continuous medium supply, automated sampling and real-time sensing, and precise controls of culture conditions, including physiological and mechanical forces, for long-term culture of organoids and embryoids.

Conclusions and future directions

Over the last two decades, a vast array of microfluidic technologies has been developed, with some of them even targeting single-cell and single-molecule analyses [59]. For more detailed discussions on available microfluidic technologies for bio-related applications, readers are directed to some excellent recent reviews [60,61]. Microfluidic tools compatible with mammalian cell cultures are particularly attractive for the development of next-generation embryoid and organoid cultures. Since such efforts are still at exploratory stages in research laboratory settings, polydimethylsiloxane (PDMS)-based microfluidic technologies, such as those based on soft lithography, remain the most versatile and popular ones given the compatibility of PDMS with rapid prototype device fabrication, mammalian cell culture, and live imaging. Nonetheless, changes in device material, surface coating, cell number per unit surface area, or per unit medium volume may all affect the outcome of otherwise-standard embryoid or organoid protocols that have been established using conventional culture tools. Spatial constraints in microfluidics might also present a physical limitation for long-term cultures of embryoids and organoids. Thus, it is important to fully characterize and optimize microfluidic embryoid and organoid development protocols. Future directions in this area include applying microfluidic innovations to obtain embryoid and organoid systems with enhanced maturity, functions, complexity, structural fidelity, and

disease or development relevance. Microfluidics can provide a more *in vivo*-like environment through dynamic spatiotemporal controls of chemical signals, morphogen gradients, material transports, mechanical forces, and tissue topology and orientation. The other direction is to apply microfluidics to improve the efficiency, reproducibility, and scalability of embryoid and organoid cultures, necessary for translational screens. A widely recognized challenge in embryoid and organoid cultures is the intra- and inter-batch variability. Microfluidics can reduce such variability through implementations of precisely controlled spatiotemporal signals to modulate embryoid and organoid developments.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge the support from the Michigan-Cambridge Research Initiative, the National Institutes of Health (R21 NS113518, R21 HD100931, R21 HD105126, R21 HD105192, R21 HD109635, and R01 GM143297), the National Science Foundation (CMMI 1917304, CBET 1901718, and PFI 2213845), and the 21st Century Jobs Trust Fund received through the Michigan Strategic Fund from the State of Michigan (CASE315037).

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- •• of outstanding interest

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