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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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 embryonic events, from ex utero 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 embryos 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 extra-cellular 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. Oftentimes, 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,
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 EVT-s and endothelium cells in 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 embryo 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...
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].
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 inter-connections 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
controlled positioning and orientations of organoids and embryos 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 embryos.

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.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


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Early embryonic development models


Zeng et al. developed a microfluidic postimplantation amniotic sac embryo with high efficiency and controllability, by using microfluidics to direct formation of HPSC clusters in prescribed locations before asymmetric morphogen stimulations to drive PASE formation.


