Review Article

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Microengineered organoids: reconstituting organ-level functions *in vitro*

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In vitro miniaturized organoids are innovative tools with varying applications in biomedical engineering, such as drug testing, disease modeling, organ development studies, and regenerative medicine. However, conventional organoid development has several hurdles in reproducing and reconstituting organ-level functions *in vitro*, hampering advanced and impactful studies. In this review, we summarize the emerging microengineering-based organoid development techniques aiming to overcome these hurdles. First, we provide basic information on microengineering techniques, including those for reconstituting organoids with organ-level functions. We then focus on recent advances in microengineered organoids with better morphological, physiological, and functional characteristics than conventionally developed organoids. We believe that microengineered organoids possessing organ-level functions *in vitro* will enable widespread studies in the field of biological sciences and have clinical applications.

Keywords: Engineering; Organoids; Stem cells; Culture techniques; *In vitro* techniques

Introduction

Organs consist of tissues with different cells and undergo a series of complex events involving various molecular regulatory and cellular interactions and signaling pathways [1]. Therefore, it is difficult to unravel the complete mechanisms underlying organ injuries and diseases [2]. Various methods, including tissue engineering, organ transplantation, and animal testing, have been introduced to improve the quality of human life [3–6]. Amidst these efforts, organogenesis combined with microengineering technology has been the subject of intense investigation, replacing and improving traditional methods [7]. It has taken decades to recapitulate the key aspects of organs *in vitro* [8,9]. Organoids are miniaturized organs grown *in vitro* with multicel-

lular structures exhibiting self-organization and specific differentiation upon the addition of specific growth factors [10,11]. The most important characteristic of an organoid is its ability to self-organize, which occurs through spatially restricted lineage commitment and cell sorting, and strongly depends on the activation of various signaling pathways mediated by intrinsic cellular components or extrinsic environments, such as the extracellular matrix (ECM) and culture medium [12–14]. The era of organoids began in 2008, when Eiraku et al. [15] first proposed embryonic stem cell (ESC)-based optic cup-like structures of cortical tissues *in vitro*—a milestone in the field of regenerative medicine.

Organoids maintain interactions between ectodermal, mesodermal, and endodermal layers essential for cell differentiation and tissue morphogenesis [16]. The resulting mesoderm surrounds the epithelium of the developing organoids and differentiates into fibroblasts and smooth muscles [17]. Pluripotent stem cells (PSCs), ESCs, or induced PSCs (iPSCs) are used to form organoids from three different lineages: ectodermal, mesodermal, and endodermal. Ectodermal lineages form specific neural structures within the nervous system or 3-dimensional (3D) sensory nerves by introducing multiple signaling pathways, such as wingless-related integration (Wnt) site, fibroblast growth factor (FGF), bone morphogenetic protein (BMP), retinoic acid, and sonic hedgehog signaling pathways [18,19]. The mesodermal lineage forms nephrons through the transformation of the metanephric mesenchyme derived from the intermediate mesoderm and the associated signaling pathways, such as sine oculis-related homeobox, homeobox protein, cadherin-associated protein beta, Wnt, FGF, and BMP signaling pathways [20]. The endodermal lineage forms the foregut, midgut, and hindgut by inducing activin/nodal, Wnt, FGF, and BMP signaling [21–23]. Many studies have attempted to standardize the protocol for developing microengineered organoids from the three primary lineages, including the ectodermal lineage for brain [24] and inner ear organoids [25], the mesodermal lineage for kidney [26], heart [27], bone [28], and skeletal muscle organoids [29], and the endodermal lineage for lung (foregut) [30], liver (foregut) [31], intestine (midgut) [32], and colonic organoids (hindgut) [33].

Similarly, chemical growth factor-induced approaches and bioengineering methods recapitulating the functions of organs also contribute to the study of human organ development and diseases in a dish. Traditional culture platforms for organoid formation face difficulties in controlling uniform aggregate formation, and their expanded utilization has been hindered by limitations, such as a lack of high-fidelity cell types, atypical physiology, and limited maturation [34]. The current culture systems also have a limited ability to develop organoids with a complex and dynamic microenvironment that can provide important cues for organogenesis, including a lack of absolute accuracy for cell transition and cell fate, lack of realization (development of areas with different functions), atypical physiology, and limited maturation. To address these limitations, we need to develop advanced *in vitro* techniques for organoid research. Microengineered organoid technology can be an alternative approach to achieve organ-level functions in organoids *in vitro* [35]. At the forefront of this undertaking, we describe current microengineering techniques, such as microwells, microfluidic chips, biomimetic microtopography, and microelectronic chips that provide suitable conditions for the development of complex organoids. We subsequently summarize these technique-based applications according to the organoid type (i.e., ectodermal, mesodermal, and endodermal lineages). Finally, we discuss the future perspectives of microengineered organoids to reconstitute organ-level functions and their various biomedical applications.

Microengineering to develop organoids

Ethics statement: This study was a literature review of previously published studies and was therefore exempt from institutional review board approval.

1. Microwells

Microwells produce rapid and uniform 3D cell aggregates with a natural construction and unique features of functional tissues. Unlike cells in 2-dimensional (2D) cultures, 3D cell aggregates engage in induced cell-cell interactions and form organizations and the ECM more easily. Because organoid formation is a nonlinear deterministic system, the initial aggregate formation is important. Shin et al. [36] first developed microwells to support the organization of salivary gland stem cells, demonstrating phenotypically and functionally enhanced salivary structures, such as salivary gland epithelial tissues. Compared with traditional organoid techniques, such as Matrigel embedding or floating cultures, the proposed square microwell showed better efficiency in 3D organization in terms of tissue structure and function. The use of microwells for organoid development started in 2020. To date, intestinal, cerebral, lung cancer, kidney, liver, retinal, blood-brain barrier, and pancreatic duct-like organoids have been developed. Wiedenmann et al. [37] designed a microwell chip to generate pancreatic duct-like organoids composed of

four hexagonal arrays with 12 round pillars surrounding each array (diameter: 150, 300, and 600 μm), using soft lithography (Fig. 1A, upper images) to maintain an aqueous drop (20–40 μL) through surface tension and to ensure homogeneous and efficient cell seeding. They controlled the 3D cell aggregate diameter by adjusting both the cell number and well diameter and developed the final products of a microwell chip with 1196 wells containing 0.7 mL of secretome culture medium. Brandenberg et al. [38] proposed a high-throughput automated organoid culture in microengineered cell culture devices called microcavity arrays (diameter: 400 and 800 μm) (Fig. 1A, bottom images). U-shaped microwell arrays generated on a silicon substrate using soft lithography were transferred from a polydimethylsiloxane mold to the hydrogel surface on the dish. The authors established a method for automated high-content phenotypic drug screening. The automated and manual processes exhibited similar stem cell colony-forming efficiencies. The same group reviewed the importance of controlling the initial culture conditions, such as the microenvironment, in steering cellular development and asserted that a small variation in the initial stage can dramatically influence the final organoids because organoid formation is a nonlinear deterministic system [39]. Microwells are known to provide a realistic and controlled 3D microenvironment for cells, leading to spheroids with controlled sizes. In a monolayer culture system, cells are present in a single space, making individual assessment difficult. Therefore, microwells overcome this by isolating single cells at specific sites. Another advantage is the ease of medium changes and integrated techniques such as automated fluorescence microscopy and plate readers. Manuel et al. [40] recently constructed protein microarrays with microwells, which were convenient for several biological assays. However, there are a few restrictions in the direct usage of microwells, such as the unstable morphology of organoids and a lack of background knowledge on cell fate decisions. The microwell is still an efficient tool for controlling the initial structure of 3D cell aggregates. However, the final products of a few organoids, including inner ear organoids, are as small as 2 mm. Therefore, the scale and structure of the microwell must be considered to improve organoid development efficiency.

2. Microfluidic chips

Yu et al. [41] summarized the necessity of applying a microfluidic system for the development of organoids as follows: (1) to recapitulate the complex microenvironment and multiple cell types; (2) to facilitate the exchange of nutrients, waste, and gas; and (3) to form standardized organoids with reproducible mass production of homogeneous organoids. Microfluidic systems are efficient tools for improving the development of complex organoids with reconstituted organ-level functions. They enable controlled fluid flow, co-culture of different cell types, and the formation of diverse matrices [42]. Microfluidic systems can be classified as direct or indirect. A direct microfluidic system offers flow through a microchannel via a pump, and an indirect one generates flow from the external movement of the shaker platform at a low speed. Achberger et al. [43] introduced a microfluidic chip for the development of self-forming retinal organoids derived from human ESCs or human iPSCs by combining the retinal pigmented epithelia (Fig. 1B, upper images). After culturing the retinal pigmented epithelia, the retinal organoids were embedded in a hydrogel, and the medium was perfused at a constant rate under the retinal pigmented epithelium on the porous membrane with a pore diameter of 3 μm and a thickness of 10 to 20 μm. The interaction between retinal organoids and retinal pigmented epithelium enhances the formation of the outer segment of retinal organoids, enabling the modeling of key functionalities of the visual cycle, such as the calcium flux [44]. To generate functional liver organoids, Jin et al. [44] proposed a pump-free microfluidic device. Under a physiologically relevant culture microenvironment, the liver organoids showed improved hepatic function, metabolic activity, biosynthetic activity, and drug responses. The proposed microfluidic device enabled the co-culture of intestinal and stomach organoids. The authors also revealed interactions between these organoids. In addition, the same group investigated the microfluidic device-induced development of brain organoids under fluid flow conditions, with precisely controlled fluid flow and effective exchange of oxygen, nutrients, and bioactive molecules in the medium, leading to robust expansion and reduced cell apoptosis at an early stage of organoid development [45]. The brain organoids in this indirect microfluidic system have the advantage of improved cortical layer development, volumetric augmentation, and electrophysiological properties and have been applied as an effective modeling platform for drug testing (Fig. 1B, bottom images) [46]. The application of microfluidic chips has many benefits, such as requiring smaller sample size and reagent volume, which makes the method more cost-effective. Due to their high-throughput nature, microfluidic chips allow heterogeneous systems to be analyzed with great precision. The usage of pumps, tubing, connectors, and valves can serve as a limitation, but microfluidic systems ensure a continuous supply of medium, improving the functions of organoids. Hence, the culture conditions of microfluidic systems, including flow rate and medium concentration, should be taken into account under various conditions. A multi-organoid on a chip was recently introduced,

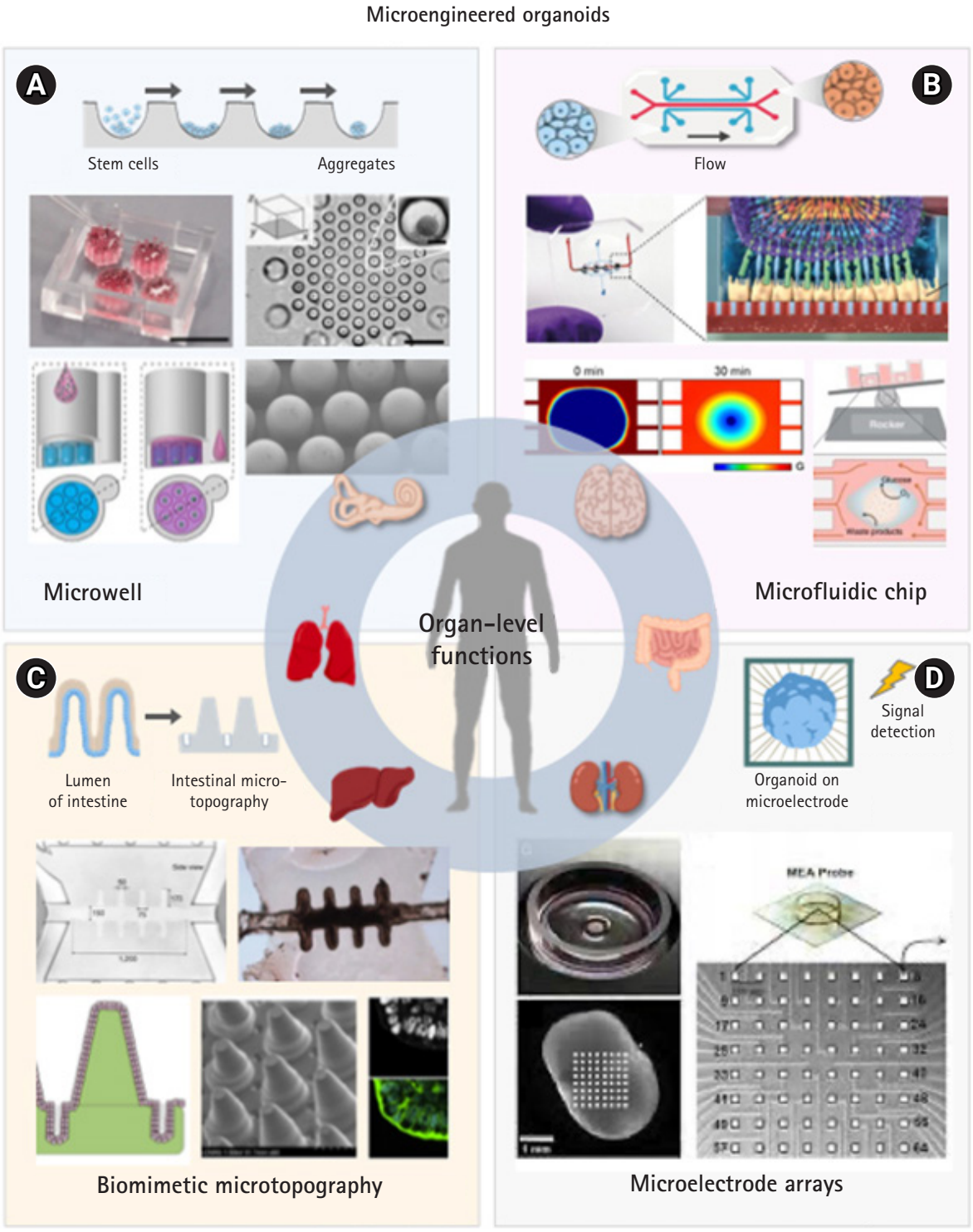

Fig. 1. Representative microengineering to reconstitute organ-level functions of organoids. (A) Microwell arrays to form aggregates with high uniformity and reproducibility. Adapted from Wiedenmann et al. Nat Biomed Eng 2021;5:897–913, with permission from Springer Nature [37]. Adapted from Brandenberg et al. Nat Biomed Eng 2020;4:863–74, with permission from Springer Nature [38]. (B) Microfluidic chip to provide a supply of nutrients and co-culture with different cell types. Adapted from Achberger et al. Elife 2019;8:e46188 [43]. Adapted from Cho et al. Nat Commun 2021;12:4730, with permission from Springer Nature [46]. (C) Biomimetic microtopography that can form specific organ-mimicking microstructure. Adapted from Nikolaev et al. Nature 2020;585:574–8, with permissions from Springer Nature [47]. Adapted from Creff et al. Biomaterials 2019;221:119404, with permissions from Elsevier [49]. (D) Microelectrode arrays to detect the electrophysiology of organoids with electrogenic cells. Adapted from Fair et al. Stem Cell Reports 2020;15:855–68, with permissions from Elsevier [51].

which is encouraging for the microfluidic chip-based development of functional organoids and their advanced applications.

3. Biomimetic microtopography

It is difficult to form homeostatic and functional structures using intestine-derived stem cell-based epithelial organoids in 3D matrices because they are difficult to manipulate experimentally. To maximize the intrinsic self-organization properties of cells, capillary force lithography and 3D printing techniques are typically applied to fabricate tissue-mimicking scaffolds with microtopography. Nikolaev et al. [47] developed a tube-shaped microfluidic device mimicking tubular guts that can form the lumen and crypt- and villus-like domains (Fig. 1C, upper images). Through perfusion in a microfluidic device, the lifespan of the intestinal tissue was extended by removing dead cells. The authors also reported epithelial wound healing after damage by targeted laser ablation using a mini-gut tube. The same group recently proposed deterministic organoid patterning via external cell regulation, with a heavy emphasis on the final functional architectures of real organs—the production of both epithelial self-organization and extrinsic microenvironmental controllers [48]. Using an elastomeric stamp, microwells with rod-like geometry were generated on the hydrogel, and dissociated intestinal cells were cultured on it. Through an image analysis of high-throughput segmentation, they demonstrated the cell shape-mediated regulation of signaling pathways in the patterned intestinal organoids. Specifically, the prepatterned epithelial cells showed different yes-associated protein 1 and Notch activities, changing the crypt- and villus-like domains by promoting Paneth cell differentiation and suppressing stem cell fates. In addition, the authors confirmed intestinal epithelial cell death and extrusion from the epithelium for the first time, which has remained a topic of interest for *in vitro* systems. Apoptosis occurs in micropatterned intestinal organoids, followed by shedding *in situ* before the extrusion of dead cells. Later, Creff et al. [49] developed a 3D scaffold to reproduce the intestinal epithelium topography using 3D stereolithography with high resolution (Fig. 1C, bottom images). To determine the optimal conditions of the intestinal tissue-inspired scaffold, various mixtures were tested with SW480 colorectal cancer cells, and the 40% polyethylene glycol diacrylate+30% acrylic acid+laminin condition was selected for the study. Caco-2 cells were cultured on a fabricated scaffold and villi formed as observed in the body, revealing the promotion of cell differentiation and organization after the introduction of 3D stereolithography-based intestinal tissue-inspired scaffolds. Until now, complex intestinal tissues were mostly mimicked using microengineering. Recently, it has been proven that topography is one of the most crucial physical cues for stem cells. Surface topography provides greater surface roughness, which thereby enhances surface moisture retention. Nonetheless, artificial scaffolds may come with some limitations such as the polymer used, as biomaterials need to be modified in terms of surface energy and chemical compo- sition, which directly affects the cellular response. In some cases, the by-products of nanofibrous scaffolds may also affect cell migration and proliferation. Since the human body consists of many complex tissues and organs, more in-depth studies testing various technologies are needed.

4. Microelectrode arrays

Microelectrode arrays (MEAs) represent one of the major microphysiological systems (MPSs) designed to investigate the function of the nervous system, including the peripheral and central nervous systems (CNSs) [50]. MEAs can record and simulate the activity of electrogenic cells, including neurons and hair cells. Unlike traditional MEAs, which detect signals from cultured monolayer cells, current MEAs can work with 3D organoids. MEAs have been applied to organoids related to the ectodermal lineage, including sensory systems such as brain and inner ear organoids. Fair et al. [51] quantified the electrophysiology of cerebral organoids using MEAs and correlated it with molecular and cellular development (Fig. 1D). To apply MEAs to cerebral organoids, the authors established a 64-channel MEA platform to record the extracellular field potential charge activity and check the spontaneous electrical activity without invasion. These MEAs measured the electrical activity from the surface layer cells and cells present three layers deep via a non-destructive method. Furthermore, McDonald et al. [52] developed a hammock-like mesh MEA by embedding microelectrodes, in which neural organoids could grow along the polymer filaments. The electrical activity in the neural organoids was detected at each electrode through the titanium-gold-titanium electrical paths, showing a single electrode trace. The authors confirmed the electrophysiology for 35 days and demonstrated that the signal amplitudes were reduced by increasing the distance between the electrodes and active cells. To cover the 3D shape of organoids, Huang et al. [53] fabricated miniaturized chip-integrated MEA caps inspired by the shape of electroencephalography caps. They are composed of self-folding polymer leaflets and conductive polymer-coated metal electrodes and exhibit an optically transparent structure. This structure can detect the electrophysiology of organoids with different sizes from 400 to 600 μm for up to 4 weeks. The authors investigated the response after glutamate treatment and

confirmed an increased signal-to-noise ratio. MEA systems have the potential to easily record neural networks, as well as cardiac excitability and contractibility. Despite being an effective tool for sensing electrogenic cells in organoids, they still present some limitations; for instance, the initial MEA insertion causes damage to the blood-brain barrier and causes dysfunction of the CNS. Therefore, more sophisticated detection methods are needed for in-depth analyses.

Microengineered organoids with reconstituted organ-level functions

1. Ectoderm

1) Brain organoids

The CNS is a complex arrangement of glial, immune, and neuronal cells consisting of discrete areas responsible for functions such as motor control, sensory information processing, and memory. To recapitulate the functions of the brain, microengineered brain organoids have been developed [54]. Because 2D and 3D models were unable to mimic the physiology of the native CNS in terms of the fluidic conditions and mechanical properties, brain organoids developed using bioengineering approaches were generated using human iPSCs. To create more complex microengineered brain organoids that can mimic the native environment of the human brain and important interactions that occur in it, such as progenitor cell interactions, neuron-to-neuron cell interactions, astrocytic-neuronal interactions, microglial-neuronal interactions, oligodendrocyte-neuronal interactions, and vascular cell interactions, multiple protocols have been standardized [55]. Lancaster and Knoblich [56] established the first protocol for the growth of human cerebral organoids using a Matrigel matrix to assist in organoid formation. The developed cerebral organoids showed major brain features, such as apical-basal polarity, division modes of neural stem cells, and neural migration. Brain organoid generation can be achieved via two methods: self-patterning (relying on the ability of intrinsic self-organization that eventually generates whole-brain organoids) and pre-patterning (relying on the use of extrinsic growth factors or small molecules to promote differentiation towards specific brain regions) [57]. MPSs have been considered for the development of brain organoids, as they can replicate the electrophysiological properties of the brain. An MPS differs from basic cell culture systems in that it can mimic organ-level functionality, including the overall architecture and cell-cell interactions. These include brain organoid-on-a-chip models based on microfluidics [58], microtopographic scaffolds [59], 3D-printed microwell arrays [60], and MEAs to study the electrical activity of organoids [61]. Whitesides et al. [62] pioneered soft lithography that enabled organoid formation in a physically confined microenvironment, such as microchannels. Park et al. [63] demonstrated a microfluidic co-culture platform for CNS research. Booth and Kim [64] generated a porous membrane-based microfluidic model so that cells could communicate with each other in a cytokine-mediated manner. MEA models have been beneficial for studying the neuronal electrical activity of brain organoids [65]. For example, MEA plates can be used to measure electrical activity with the help of electrodes, nanowires, and 3D nanostructures, enabling high-speed recording and stimulation of iPSC-derived brain organoids (Fig. 2A) [53]. Altogether, we can conclude that studying physiological systems with 3D organoid models is better than studying monolayer systems from a translational perspective [66].

2) Inner ear organoids

The inner ear is a complex system composed of sensory epithelial cells, including hair cells, spiral ganglion neurons, cochlear spiral ligaments, and the stria vascularis. To generate an inner ear model through microengineering, the early developmental processes must be considered. Many studies have reported that inner ear cells can be generated from three different origins: the otic placode, neuroepithelial cells, and the neural crest [67]. In 2014, Koehler and Hashino [68] provided some insight into inner ear organoid modeling by developing a 3D culture protocol to generate ESC-derived inner ear organoids containing sensory epithelial hair cells. In 2018, Jeong et al. [69] used this protocol and combined it with their own strategy to generate a human inner ear organoid model containing cochlear hair cells. To achieve a functional model of an inner ear organoid, cells need to be arranged in a spatiotemporal manner [70]. Reciprocating micropumps of microfluidics and microelectromechanical system technologies have recently been reported as methods for safe and effective drug delivery, allowing direct injection into the inner ear. Mattei et al. [71] illustrated a dynamic 3D rotatory cell culture system for deriving inner ear organoids from iPSCs. This group recapitulated the inner ear developmental stages, thereby giving rise to hair-like cells and accessory otoconia-like structures (Fig. 2B). Although the development of inner ear organoids has a long history, microengineering-based progress has been painfully slow compared to that of other organoids. Major breakthroughs have been made in inner ear modeling, but current models are limited in their applications for translational research.

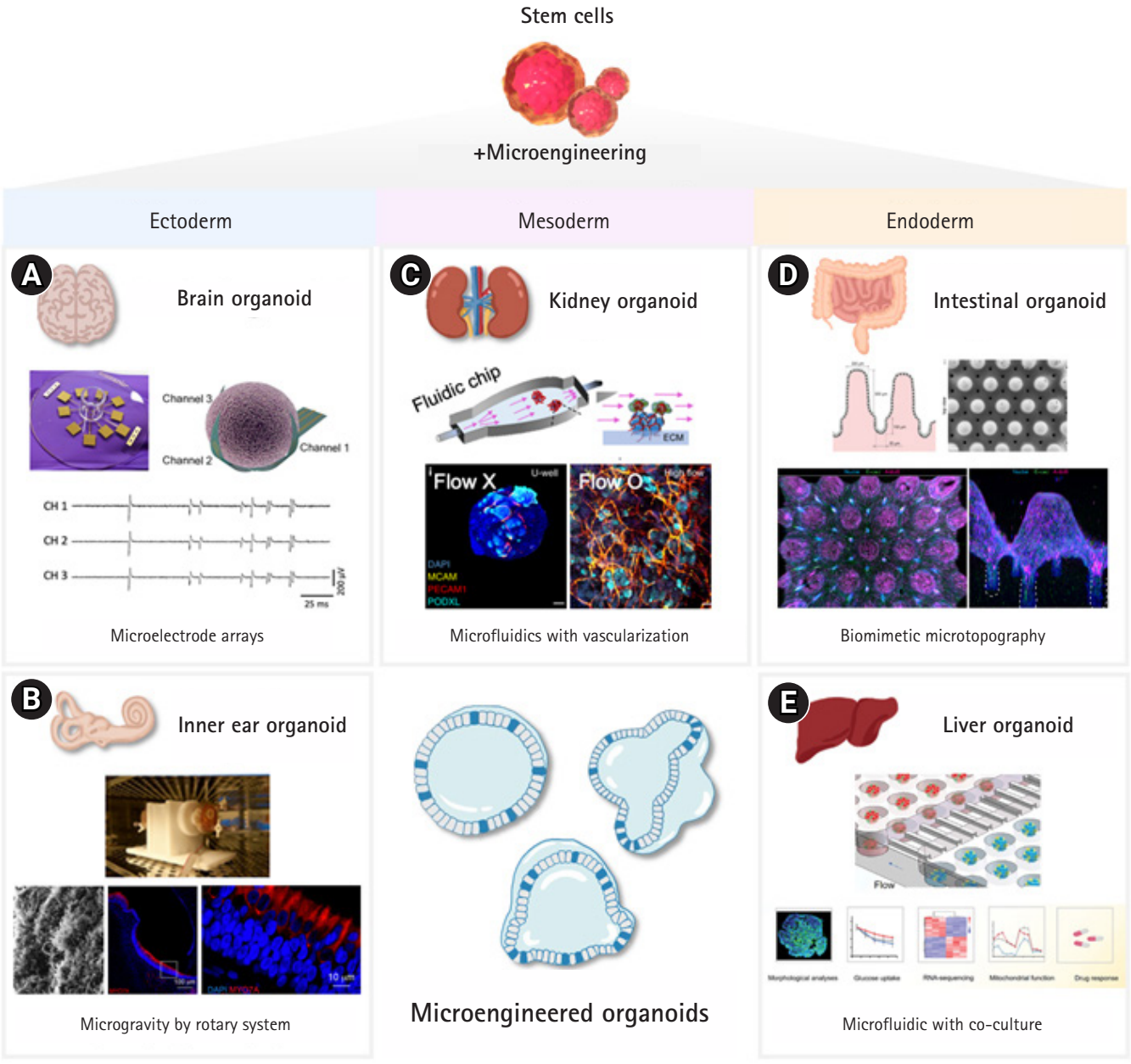

Fig. 2. Microengineered organoids with reconstituted organ-level functions. (A) Microelectrode array-based versatile recording from human induced pluripotent stem cell-derived brain organoids. Adapted from Huang et al. Sci Adv 2022;8:eabq5031, with permission from Science Advances [53]. (B) Microgravity by rotary systems to improve the maturation of human pluripotent stem cell (hPSC)-derived inner ear organoids. Adapted from Mattei et al. Front Cell Dev Biol 2019;7:25, with permission from Frontiers [71]. (C) Microfluidic chip-based vascularization of hPSC-derived kidney organoids. Adapted from Homan et al. Nat Methods 2019;16:255–62, with permission from Springer Nature [77]. (D) Intestine-mimicking microtopography that provides the complex microstructure of the intestinal surfaces to mouse intestinal crypt-derived intestinal organoids. Adapted from Gjorevski et al. Science 2022;375:eaaw9021, with permission from The American Association for the Advancement of Science [48]. (E) Microfluidic chip with co-culture of human induced pluripotent stem cell-derived liver and islet organoids. Adapted from Tao et al. Adv Sci (Weinh) 2022;9:e2103495, with permission from Advanced Science [93].

2. Mesoderm

1) Kidney organoids

Each human kidney is composed of approximately one million nephrons that filter blood and maintain electrolyte homeostasis by reabsorbing the necessary nutrients back into the blood [72]. Since chronic and acute kidney injuries are on the rise owing to the increased use of prescription drugs, there is an

urgent need for the development of microengineering-based 3D models [73]. Kidney organoids generated using microengineering approaches have been developed that reproduce normal kidney functions by replicating specific regions of the nephron, including podocytes in glomerular capillaries or epithelial cells in proximal tubules, loops of Henle, and distal convoluted tubules [74]. Kidneys contain at least 26 interacting cell types, and such complex environments are difficult to recapitulate in 2D cultures. Hence, 3D models with MPS are needed to study cellular responses, biochemical processes, and responses in the presence of multicellular interactions [75]. Researchers developed a 3D proximal-tube-on-chip model derived from kidney organoids using a microfluidic platform with integrated gelatin-fibrin ECM. The proximal tube, which is the first segment of the tubular network of the nephron, is responsible for nutrient absorption, making it highly susceptible to damage by drugs and toxins. Chapron et al. [76] applied the same renal proximal tubular epithelial cell (PTEC) microfluidic platform to demonstrate the significance of renal megalin (an endocytic receptor) in vitamin D homeostasis. They also reported the development of a microfluidic system with dual channels for co-culturing human PTECs with human umbilical vein endothelial cells to generate a vascularized kidney proximal tubule model. A study also reported a microfluidic device with an "interstitial" matrix that facilitates solute travel between a vascular endothelial channel and a tubular epithelial channel, thereby showing more mature vascular structures (Fig. 2C) [77]. Another study reported the fabrication of a kidney organoid-on-chip system with microwells made using a 3D printer to cultivate 300 to 500-μm-long organoids per microwell [78]. The development of such 3D cell culture technologies combined with microengineered platforms provides an optimistic outlook for the treatment of acute and chronic kidney diseases while reducing the use of animal testing [79].

3. Endoderm

1) Lung organoids

Lung organoids derived from iPSCs were first reported in 2015 by the Dye et al. [80]. Human iPSC cultures are directed towards the endodermal lineage using activin A, mimicking Nodal signaling. There have been many studies on the generation of microengineered lung organoids. Despite overcoming many disadvantages, conventional organoid models still present several limitations. Therefore, advanced microengineering-based technologies are required. Recently, many scientists have been working on microengineered lung organoid-on-chip models that can mimic the *in vivo* tissue microenvironment by ensuring the controlled flow of nutrients and imitating breathing-like motion in the lung [81]. Several models have been developed over the past years using PSCs and multi-channel microfluidic platforms to enable the formation of lung organoids phenotypically similar to the lungs. Moraes et al. [82] developed a small human airway-on-a-chip model that exhibited impressive structural integrity and barrier function. The culture system was integrated with a microfluidic plug generator that created microliquid plugs mimicking the closing and opening of the airways in diseased lungs, thereby proving that plug rupture can cause damage to the lung epithelium. Since lung cancer remains the leading cause of cancer-related deaths worldwide, microengineered lung cancer organoids (LCOs) are becoming popular among oncologists. One research group developed an integrated superhydrophobic microwell array chip for high-throughput 3D culture and analysis of micro-LCOs [83]. Another study demonstrated the development of microfluidic platform-based LCO culture that enables the loading, expansion, and evaluation of drug responses under physiologically relevant flow conditions [84]. This allowed a stable flow of nutrients and oxygen to the organoids and easy drug delivery through diffusion. Altogether, microengineered lung organoids hold great potential for investigating lung regeneration and diseases.

2) Intestine organoids

The human intestine hosts the gut microbiome, which interacts with the lymphoid tissues and the immune system, exhibiting a complex microvilli structure. Current approaches to research on the intestine involve colorectal cancer cell lines, which are highly proliferative and easy to use. Epithelial cell lines such as Caco-212 and Ht-2915 have been shown to provide a significant intestine cell culture system when seeded on ECM-coated membranes. Although these 2D systems are commonly used, they fail to recapitulate the native 3D tissue morphology and key functions, such as villi formation, cytochrome-450-based drug metabolism, and mucus production. Microengineering has brought about a new way to study the human intestine [85]. It is technically challenging to culture primary human intestinal epithelial cells. However, they spontaneously undergo villus-crypt morphological organization when seeded on an ECM-coated 3D gel. Intestinal organoids lack the supporting cells found *in vivo* and experience fluid flow different from that in the native environment, making it difficult to control nutrient uptake and the microbiota [86]. To overcome these challenges, many researchers have introduced microengineering techniques to develop intestine-on-chip models. To better understand the 3D

Table 1. Microengineering approaches to reconstitute organoids with organ-level functions *in vitro*

Lineage	Organoid	Approaches	Cell type	Features/scale	Ref.
Ectoderm	Brain	Microwell	iPSCs	3D printed microwell arrays: ~800 μm (flat/curved surface)	[40]
			hiPSCs	Micropillar array	[60]
			hESCs	For imaging, the generated organoids were transferred to a microwell of flat cones/grid cones/suspended grid	[56]
		Microfluidic chip	iPSCs	Pump-free microfluidic device 3D dynamic culture	[24]
			iPSCs	Co-culture of brain organoids with vascularization	[63]
		Biomimetic microtopography	iPSCs	Two fiber substrates with diameters of 1.25 and 3.23 mm for organotypic cell culture	[38]
				Matrigel-alginate composites	[39]
		Microelectrode arrays	iPSCs	3D shell MEAs with 400- to 600-mm organoids	[53]
				Cerebral organoid connectivity apparatus with a spiked and grid design	[61]
			iPSCs and hESCs	1,024 Recording electrodes	[65]
	Inner ear	Microfluidic chip	hiPSCs	Electrospray microcapsules	[70]
				Diameter: ~300 μm	
				Shell thickness: ~50 μm	
				Rotary cell culture system	[71]
Mesodermal	Kidney	Microwell	hPSCs	384-well organoid plate	[73]
			hiPSCs and ESCs	Diameter: 100 and 600 μm	[78]
				Height: 2 and 2.1 mm	
		Microfluidic chip	hPSCs	Diameter 100–380 μm within the multiplexed chip device	
				Full factorial microbioreactor array with perfusion system	[77]
		Biomimetic microtopography	hiPSCs	Kidney decellularized ECM hydrogel	[75]
Endodermal	Lung	Microwell	Lung tumor cells	A superhydrophobic microwell array chip with a 12×9 microwell array of superhydrophobicity (diameter: 200 μm)	[80]
				Superhydrophobic microwell array chip with 108 microwell	[84]
				Diameter: 1.37 mm	
				Depth: 300 μm	
		Microfluidic chip	Lung tumor cells	Each microphysiological system device with 29 wells	[82]
				Depth: 750 μm	
				Width: 500 μm	
	Liver	Microwell	iPSCs	A microwell plate with 7,000 well and liver-ECM-derived microparticles	[90]
				Diameter: 400 μm	
		Microfluidic chip	iPSCs	Two chambers (height: 800 μm, width: 1.5 mm, length: 1.75 mm), each chamber with 240 microwells (diameter: 500 μm, depth: 800 μm). The culture compartments were connected by microchannels (height: 100 μm, width: 100 μm, length: 250 μm).	[93]
		Microfluidic chip	HepaRG cell line	Bird's-eye view of three different multi-organoid chips	[94]
		Biomimetic microtopography	Mouse liver progenitor cells	Poly(ethylene glycol) hydrogels with stiffness ≈ 1.3 kPa with incorporated ECM proteins	[91]
	Intestine	Microwell	PSCs	Microwell arrays with 289 cylindrical holes	[96]
				Diameter: 500 μm	
				Depth: 300 μm	
		Microfluidic chip	hiPSCs	Porous (7 mm) polydimethylsiloxane membrane coated with Matrigel with/without flow	[88]
			Human intestinal epithelial Caco-2 cells	Basement membrane with/without flow	[89]
				Height of upper and lower microchannels: 500 μm and 200 μm, separated by a 20-μm porous membrane	[47]

iPSC, induced pluripotent stem cell; 3D, 3-dimensional; hiPSC, human induced PSC; hESC, human embryonic stem cell; MEA, microelectrode array; ECM, extracellular matrix; hPSC, human PSC.

structure of an intestine, microfluidic devices and micromolding methods are being used to create a villus-like morphology. Kim et al. [87] described a more sophisticated gut-on-chip model created with a microfluidic device that enables human intestinal cells, such as epithelial cells, immune cells, and microbial cells, to proliferate and experience physiological fluid flow. To add to it, Gjorevski et al. [48] reported the microfabrication of intestinal organoids using microstructured 3D hydrogels with cavities of defined size and shape to produce organoids with controlled geometry (Fig. 2D). Another study demonstrated the use of polymer film-based microwell arrays to culture intestinal organoids. Moreover, Shin et al. [88] illustrated the fabrication of a microfluidic gut-on-chip model that enabled the independent control of fluid flow, thereby influencing intestinal morphogenesis. A study showed the influence of microwell biomimetic topography on the intestinal epithelial Caco-2 cell phenotype. They reported that these microwell structures exerted mechanical stress to help the cells migrate and spread, which in turn regulated cell proliferation, differentiation, and survival [89]. Ultimately, the use of microengineered intestinal organoids rich in microbiota and immune cells will help us understand disease modeling and create personalized medicine.

3) Liver organoids

Microengineered liver-specific organoids can be derived from PSCs or liver-specific stem/progenitor cells [90]. The generation of hepatic organoids from iPSCs was first proposed by Takebe and colleagues using an elegant co-culture model. The group showed the generation of 3D aggregates, known as iPSC liver buds, embedded in Matrigel culture [91]. Before the era of organoid technology, many researchers attempted to apply bioengineering approaches to reconstruct the native liver microenvironment. This idea eventually gave rise to microengineering-based approaches, such as microfluidic culture systems, manipulation of cell culture matrices, microwell arrays, and the design of liver organoid-on-chip platforms. These strategies have been used to create different bioengineered 3D liver culture systems. For example, Sorrentino et al. [92] created mechano-modulatory synthetic niches for liver organoids to mimic their native microenvironment. Another study demonstrated a multi-organoid-based system by co-culturing liver organoids and islet organoids on a microfluidic chip. This system enhanced cell viability and growth (Fig. 2E) [93]. Over the past few years, microengineering and organoid technologies have opened new avenues for understanding the native microenvironment of the liver. The ability to expand the technology to create patient-specific microengineered organoids has been of great interest. The established microengineering-based liver organoid models provide an upfront solution for rare and genetic diseases, such as alpha-1 antitrypsin deficiency, hepatotoxicity, and liver cancer [94]. Over the past decade, microengineered liver organoids have become an increasingly promising option for personalized liver medicine.

Conclusion

Organoids allow the study of organogenesis and tissue regeneration. Compared with traditional 3D cell aggregation methods using ultra-low attachment plates, microengineering-based organoid formation promotes uniformity, cell-ECM interactions, differentiation, and vascularization, better recapitulating the native tissue structure and function (Table 1) [24,38–40,47,53,56,60,61,63,65,70,71,73,77,78,80,82,84,88–91,93,94,96]. Although organoids enhance the development of the desired organs, we need microengineering tools to control organoid maturation and function [95]. This review presented the current state of microengineering-based organoid technology with a focus on tissue regeneration. The term "microengineered organoids" has recently gained popularity due to its highly promising potential in traditional fields, such as tissue engineering and biomedical research [96]. We also provided an overview of the advantages and disadvantages of the previously mentioned microengineering methods.

All the techniques and associated devices discussed in this review greatly enhance the design and fabrication of more reliable models to mimic the native environment of the desired organ from all three lineages. For example, the synergistic effect between microfluidic-based devices and organoids is a promising strategy for achieving controlled fluidic interactions [97]. Similarly, microwell-based organoids form a remarkable alliance to achieve uniformity among the developed organoids, further enhancing reproducibility [98]. Although microengineered organoids have come a long way, many factors still need to be reconsidered and revised. For example, multi-organoid model systems can be further tailored to ensure a balance between stem cell differentiation of all associated organoids while meeting developmental needs at all stages. These next-generation microengineering techniques improve simulated microstructures by focusing on biocompatibility, cell attachment, and migration [99]. Apart from modeling tissue morphogenesis, microengineered organoids also allow us to study inherited diseases, which are extremely challenging to replicate in the field of regenerative medicine. Hence, introducing microengineering strategies to conventional organoid culture methods can facilitate their trans-

lation to real-life applications, thus fulfilling unmet expectations in the field of regenerative medicine.

Notes

Conflict of interest
No potential conflict of interest relevant to this article was reported.

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Data availability
Please contact the corresponding author for data availability.

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