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Advances in Microfluidic Technologies in Organoid Research

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Organoids have emerged as major technological breakthroughs and novel organ models that have revolutionized biomedical research by recapitulating the key structural and functional complexities of their in vivo counterparts. The combination of organoid systems and microfluidic technologies has opened new frontiers in organoid engineering and offers great opportunities to address the current challenges of existing organoid systems and broaden their biomedical applications. In this review, the key features of the existing organoids, including their origins, development, design principles, and limitations, are described. Then the recent progress in integrating organoids into microfluidic systems is highlighted, involving microarrays for high-throughput organoid manipulation, microreactors for organoid hydrogel scaffold fabrication, and microfluidic chips for functional organoid culture. The opportunities in the nascent combination of organoids and microfluidics that lie ahead to accelerate research in organ development, disease studies, drug screening, and regenerative medicine are also discussed. Finally, the challenges and future perspectives in the development of advanced microfluidic platforms and modified technologies for building organoids with higher fidelity and standardization are envisioned.

1. Introduction

Organoids have recently emerged as cutting-edge tools in fundamental research and translational medicine. They are self-organized 3D multicellular structures originating from human stem cells, organ-specific progenitor cells, or disassociated tumor tissues that can be cultured to resemble the original tissue upon encapsulation in a 3D extracellular matrix (ECM).[1–4] Herein, the stem cells include human embryonic stem cells (hESCs), human induced pluripotent stem cells (hiPSCs), and adult stem cells (ASCs), which self-renew and are pluripotent to generate various tissues. Organoids have been used as versatile tools to address diverse biological and clinical questions, ranging from basic stem cells and developmental biology to disease modeling, drug screening, and rational design of personalized medicine.[5–7] Compared to monolayered cells, organoids contain more diverse tissue cell types and exhibit hierarchical structures, genetic heterogeneity, cellular diversity, and cell–cell or cell–matrix interactions.[8,9] In contrast to animal models, organoids are less labor-intensive, time-consuming, and ethical and eliminate species differences from humans.[8,9] In recent years, various organoids corresponding to different tissues and organs have been established to replicate the main characteristics of human physiological pathology, such as brain,[10–14] intestine,[15–17] liver,[18,19] lung,[20] and retinal organoids.[21,22] These organoids have provided a new approach to studying tissue development, drug screening, disease modeling, and cell therapy.

In general, organoids are initiated from pluripotent stem cells that can aggregate to form embryoid bodies (EB) under 3D culture conditions, generate three germ layers (ectoderm, mesoderm, and endoderm) through induced differentiation, and eventually form organoids corresponding to specific tissues. In addition to pluripotent stem cell-derived organoids, organoids derived from tumor tissues that retain patient-specific genetic backgrounds are of great value in tumor biology research, drug testing, drug screening, and precision medicine.[23–25] However, challenges arise from high-throughput manipulation, vascularization, and controllable microenvironments in the organoid field. The lack of high-throughput manipulation has significantly limited the phenotypic screening of organoids or biomedical applications, such as drug screening. Inefficient vascularization can induce hypoxia, nutrient insufficiency, and waste accumulation in the center of organoids, thus limiting the long-term culture of tissue-scale organoids, which are expected to increase the maturity and functionality of organoids.

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Uncontrollable microenvironments, both biochemical and biophysical, have reduced the reproducibility of existing organoids, leading to poor standardization of organoid products.

Recently, microfluidic technologies have been explored in the field of organoids, usually in terms of organoid culture, formation, and functionality (e.g., vascularization). The combination of organoids and microfluidics partially addresses the challenges of existing organoids, such as dynamic 3D culture, realization of vascularization, and fabrication of functional hydrogels for organoid manipulation. The microfluidic organ-on-a-chip technology is a novel technology for controlling the microenvironment of 3D cell cultures. It can recapitulate the biochemical, mechanical, structural, and functional features of human organs by mimicking in vivo cellular microenvironment to build in vitro models with a high physiological correlation.[26–28] In addition, droplet microfluidics can be used in the fields of high-throughput screening and hydrogel fabrication because the microscale and numerous units within the microfluidic chips provide more convenience for effective manipulation.[29] Functional hydrogels produced in droplet microfluidic systems are recognized as promising scaffolds for the controllable generation and culture of organoids.[3,30]

In this review, we highlight state-of-the-art progress in microfluidic strategies that have been used to support organoid research. First, we discuss microfluidic technology for long-term and functional organoid culture with a biomimetic microenvironment, enhanced vascularization, multi-tissue interactions, and integrated biosensors. We then emphasize how hydrogels fabricated using microfluidics can be integrated into organoid systems to generate uniform and functional organoids. Subsequently, we describe typical applications of organoids in organ development, disease studies, drug testing, and regenerative medicine. Finally, we discuss the future challenges and perspectives for improving the degree of functionality and standardization to ultimately build organotypic models with higher fidelity, thus accelerating their biomedical applications.

2. Microfluidic Strategies to Enable Organoid Engineering

2.1. Design Criteria of Sophisticated Microfluidic Devices for 3D Cell Culture

In general, microfluidic devices can be tailored to various morphologies owing to the rapid development of microfabrication techniques such as photolithography, replica molding, and 3D printing. Such techniques have enabled the flexible design and customized applications of microfluidics in cell culture, especially 3D cell culture, for which the first consideration is generating and maintaining the 3D status of cultured cells. The 3D matrices, or microarrays (e.g., microwells and micropillars) have been used to introduce cellular spheroids into microfluidic devices. The former can be used to suspend and culture already formed spheroids,[31,32] whereas the latter can support the in situ formation and 3D culture of cellular spheroids, relying on geometrical guidance.[33–36] The dynamic medium is another crucial factor for 3D culture, which provides nutrients to living cells and clears metabolic wastes, simulating in vivo blood flow. To maintain a continuous flow, various driving forces, including gravity and mechanical and electromagnetic forces, have been designed and integrated into microfluidic systems. Specifically, syringes and peristaltic and pneumatic pumps are frequently used to perfuse media into microfluidic devices. In this case, the shear stress generated by the continuous flow should be optimized to a reasonable range according to the targeted cell types cultured in the microfluidic system, using simulation software (e.g., COMSOL Multiphysics) or the formula $\tau = 6\mu Q/wh^2$, where μ is the viscosity of the medium, Q is the flow rate, and w and h are width and height of the chamber,[37] respectively.

Additionally, the rational design of chips facilitates the construction of a controllable biomimetic microenvironment for 3D cell culture by creating biochemical and biophysical conditions. In general, heterogeneity is widely observed in tissues and organs in terms of the spatiotemporal distribution of cell types, biochemical factors, and biophysical signals, which can be partially replicated in microfluidic systems by generating stable biochemical or biophysical gradients. Diffusion-based gradient generators are a simple and common option for creating linear gradients in microfluidic devices. In such devices, two microchannels or microchambers filled with high and low concentrations of crucial conditions (e.g., biomedical factors and oxygen) are aligned on both sides of the cultured cells. The crucial conditions then diffuse from the high-concentration microchannel to the low-concentration microchannel to reach an equilibrium state, where the cells are surrounded by a linear gradient.[38–40] For example, Demers et al. presented a microfluidic platform capable of mimicking the spatial and temporal chemical environments during neural tube development.[39] In this study, simultaneous opposing and/or orthogonal gradients of developmental morphogens were maintained, resulting in neural tube patterning analogous to that observed in vivo. A similar strategy has been used to regulate the spatial distribution of oxygen[41,42] to modulate intracellular reactive oxygen species (ROS) levels in living cells.

Furthermore, diffusion- and convection-based gradient generator, also called "Christmas Tree" structure, within microfluidic chip is another powerful tool to modulate the signaling gradient and investigate the cellular response.[43,44] In such chips, two or more inlets are designed for injecting solutions with high and low concentrations of signaling molecules, where stable gradients can be obtained in the branched channels based on the principle of laminar diffusion and mixing at low Reynolds number. For instance, Rifes et al. developed a microfluidic chip with "Christmas Tree" structure to generate Wnt signaling gradients for modeling early heterogeneous neural tube development using hESCs, which facilitated research on the factors and processes underlying rostro-caudal neural tube patterning.[44] To control the mechanical signals applied to cells, they can be cultured on an elastomer (e.g., polydimethylsiloxane, PDMS) driven by an external force to simulate in vivo progress, such as pulmonary respiration and intestinal peristalsis.[45,46] Moreover, human body is a whole living system, and complex interactions occur among different tissues and organs, such as brain–gut axis. To replicate key tissue–tissue interactions, microfluidic chips are always endowed with compartmentalization separated by porous membranes[47–49] or micro-fences.[50,51] For example,

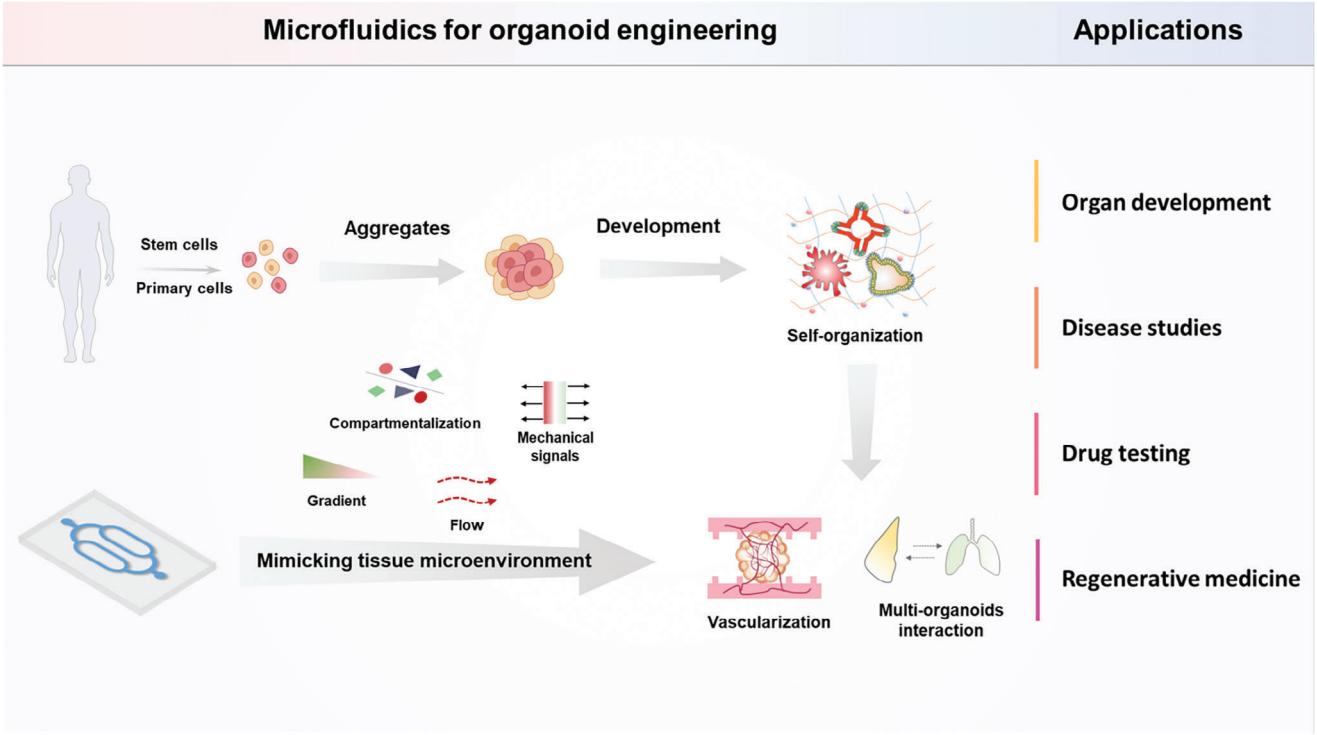

Figure 1. Schematic of engineered organoids by integrating microfluidic strategy. Organoids are 3D multicellular tissues derived from human stem cells (ESCs and iPSCs) or ASCs by self-organization. They can be engineered using microfluidic technologies by guiding stem cell differentiation, organization, and organoid formation in a controlled microenvironment, thereby improving vascularization and multiple interactions of organoids for advancing biomedical research.

Peela et al. presented a 3D microfluidic invasion platform for anticancer drug studies, which allowed for the compartmentalization of tumors and stromal fibroblasts in a defined architecture, thereby enabling pharmacokinetic drug transport to a cell-dense tumor region.[50] Depending on the above rational designs of microfluidic devices, they have facilitated dynamic culture, controllable formation, high-throughput manipulation, construction of a biomimetic tissue microenvironment, efficient vascularization, and investigation of interactions for organoids, which will be fully discussed in the following sections.

2.2. Dynamic Culture of Organoids

Compared to traditional culture systems, microfluidic chips have several advantages, such as providing a constant flow of nutrients and oxygen to proliferative organoids. These chips are usually composed of individual chambers,[52–59] sandwiched porous membranes,[47–49,60–64] or parallel aligned channels.[31,32,51,65–67] In such dynamic culture platforms, organoids undergo continuous mechanical and biochemical stimuli that regulate their growth, differentiation, and self-organization, resulting in more mature and functionally relevant in vitro physiological models[68] (**Figure 1**). Tao et al. presented a strategy for engineering human islet organoids derived from human pluripotent stem cells (hiPSCs) using a perfusable OOC platform.[33] In this study, the multilayer microfluidic device allowed the controllable aggregation of embryoid bodies (EBs) and in situ production of heterogeneous islet organoids under pancreatic differentiation conditions. The results showed that compared to static cultures, enhanced expression of pancreatic β-cell specific genes (e.g., PDX1, NKX6.1, and INS) was observed under perfused 3D culture conditions. In addition, more advanced control over organoid culture conditions can be achieved by integrating diverse sensors into microfluidic chips, including the monitoring of various essential metabolic characteristics of cells, thereby significantly improving the potential of organoids in drug screening. Dornhof et al. reported a fully integrated microfluidic platform with electrochemical chemo- and biosensor arrays to measure the oxygen, lactate, and glucose levels in a matrix-based organoid culture.[69] In this study, cell cultures were carried out in two culture compartments that closed the ECM and were separated by structured microchannels to achieve multiple microfluidic operation modes, such as medium perfusion and the introduction of external stimuli. After a week of culture, tumor organoids derived from triple-negative breast cancer stem cells showed significant differences in drug resistance compared to their 2D equivalents. Further optimization and refinement of dynamic culture systems will undoubtedly yield exciting discoveries and applications in this rapidly advancing field of research. In the following subsections, we introduce existing microfluidic strategies to enable organoid engineering, including controllable formation, high-throughput manipulation, construction of a biomimetic microenvironment, vascularization, and interactions of organoids (**Figure 2**).

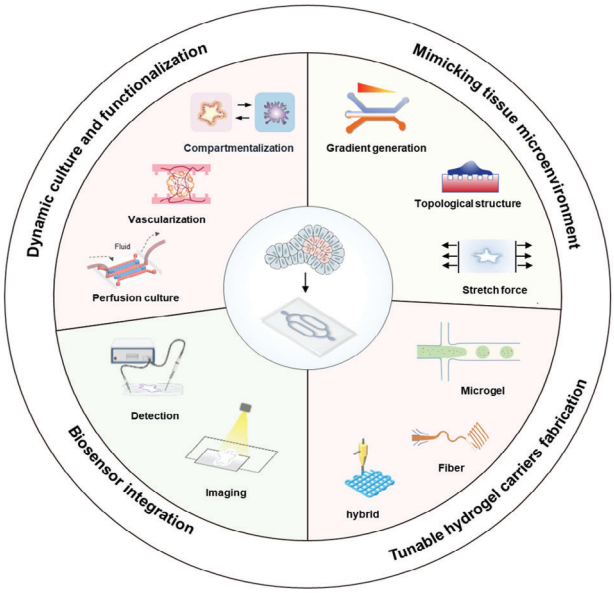

Figure 2. Overview of microfluidic technologies to support organoid research. Microfluidics has provided new strategies to accelerate the development in the field of organoids, in terms of high-throughput manipulation, fabrication of various scaffolds, and functional organoid construction.

2.3. Controllable Formation of Organoids

Massive generation of uniformly sized organoids is crucial for obtaining consistent data for drug screening and providing reliable donors for organoid-based therapy. Microwells are the most common arrays for cell aggregation and 3D culture, and the size and yield of organoids can be controlled by setting the specific parameters of the microwells. For example, Brandenberg et al. developed a strategy for high-throughput generation of gastrointestinal organoids from uniform pluripotent stem cell aggregates in a matrix-free manner.[34] As shown in **Figure 3A**, the authors fabricated PDMS stamps with micropillar arrays derived from a stiff template with U-shaped microcavities of tunable size between 10 µm and 1.5 mm. Then, non-crosslinked hydrogel solutions, such as PEG, gelatin, and alginate, were deposited onto the PDMS stamp to form hydrogel microwell arrays resembling stiff templates. Microwells have been utilized to produce and culture thousands of individual gastrointestinal organoids with a decline in the heterogeneity of the final microtissues from adult crypts, which increases the growth speed of organoids and improves their traceability for downstream analysis and accessibility. In a similar case, Wiedenmann et al. fabricated a series of hexagonal PDMS microwells with different well diameters ranging from 150 to 600 µm using 3D printing.[70] Such microwells were seeded with pancreatic progenitors derived from hiPSCs, after which chemical induction was performed to obtain pancreatic duct-like organoids. The proposed strategy facilitated the uniform aggregation of pancreatic progenitors and formation of organoids, demonstrating the potential of microwell arrays for various organoid engineering applications. To endow microwells with the ability of long-term culture and to sample the cell culture medium in real-time, perfusable microchannels can be set above or alongside the well arrays. Perfusable microwells, such as islets, liver, retina, and cancer organoids, have been designed and used for several organoid cultures and analyses.[33,46,71–77]

In addition, micropillar arrays are another simple and effective platform for high-throughput organoid production and culture and seem to be the complementary topological structure of microwells. In early studies, a sequence of micropillar chips was developed to accelerate the progress in organoid engineering.[35,36,47,78–80] In a pioneering work, Zhu et al. designed and fabricated three micropillar arrays with the same diameter of 1 mm and different intervals of 50, 100, and 150 µm.[35] Then, hiPSCs were seeded into the arrays and formed EBs in all the blank areas surrounded by four micropillars, after which massive uniform brain organoids were differentiated from the EBs via subsequent neural induction in situ. The interval between the micropillars in the arrays significantly affected the size and formation of the EBs. Generally, a large interval (100 and 150 µm) leads to the generation of larger EBs compared with that of a small interval (50 µm), but a small interval is beneficial for the production of single EBs in a blank area. Moreover, compared to microwells, micropillars are more like an open system that facilitates the diffusion of nutrients and elimination of waste within the organoids. In follow-up studies, micropillar arrays were integrated into perfusable chips for the long-term culture of organoids to investigate the effects of dynamic fluids on organoid development and functionality. Wang et al. used micropillars fabricated on a perfusable platform to engineer liver organoids derived from hiPSCs (Figure 3B).[36] This platform allows the formation of EBs, liver-specific differentiation, long-term culture, and in situ production of liver organoids. Compared to organoids cultured under static conditions, perfused liver organoids showed improved cell viability and higher expression of endodermal genes (SOX17 and FOXA2) and mature hepatic genes (ALB and CYP3A4). Other arrays such as hydrogel patterns derived from contact printing and perfused microchannel arrays fabricated by standard photolithography have also been used for the formation and expansion of different organoids, including cardiac, hepatic, and tumor organoids.[81–84]

2.4. High-Throughput Manipulation of Organoids

Generally, organoids are formed by the orderly differentiation and self-assembly of stem cells within gels (e.g., Matrigel) using Petri dishes. Such progress has limited the possibility of generating organoids on a large scale, which cannot meet the needs of high-throughput screening or regenerative medicine applications. In addition, the current differentiation protocols for various organoids are far from ideal, especially for improving their maturity and functionality (e.g., vascularization). Therefore, massive amounts of organoids must be produced to optimize organoid formation. With advances in microfabrication, various microfluidic arrays, such as microwells and micropatterns, have been developed and have emerged as promising platforms for high-throughput manipulation of organoids.

The in vivo tissue microenvironment, including biophysical and biomedical factors, is complex and dynamic, making it difficult to fully reproduce in vitro. To generate organoids with a higher similarity to their in vivo counterparts, culture conditions

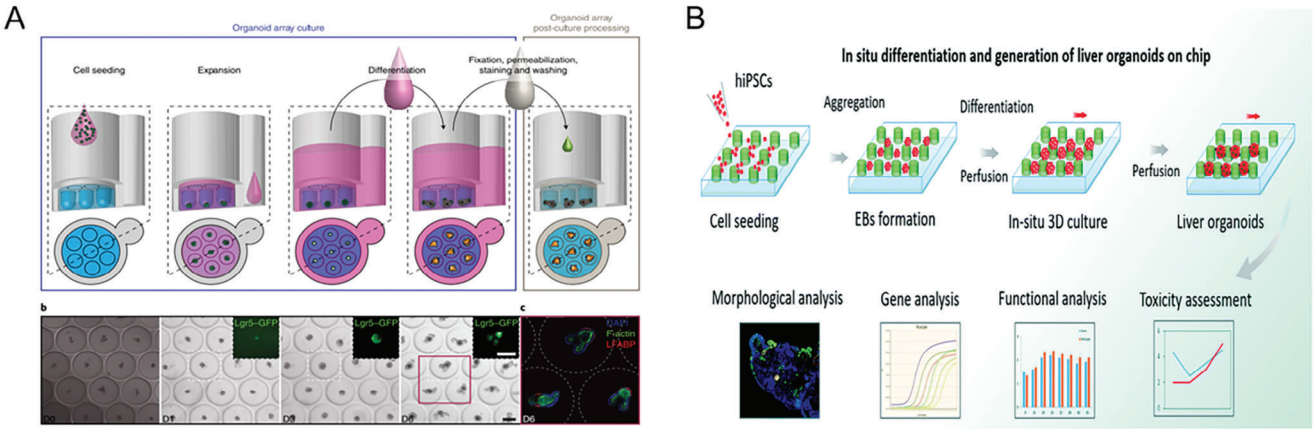

Figure 3. Representative examples of microfluidic arrays for organoid engineering. A) Microwells for uniform and massive organoid formation. In this work, hydrogel microwells with U-shaped microcavities were developed for the high-throughput generation of gastrointestinal organoids from uniform stem cell aggregates in a matrix-free manner. Reproduced with permission.[34] Copyright 2020, Springer Nature. B) Micropillars for uniform and massive organoid formation. In this work, the authors integrated micropillar arrays into perfusable chips for the formation of EBs from hiPSCs, hepatic differentiation, long-term 3D culture, and generation of liver organoids in situ. Reproduced with permission.[36] Copyright 2018, Royal Society of Chemistry.

should be systematically screened and optimized using massive organoids. Rajasekar et al. used a microfluidic platform called the IFlowPlate for colon organoid culture and vascularization under optimized conditions.[85] Specifically, the authors seeded human umbilical vein endothelial cells and primary human lung fibroblasts into gels containing 128 units of IFlowPlate to form a vascular bed under perfusion. Subsequently, patient-derived colorectal organoids were cultured on the vascular bed to further realize the vascularization of organoids by optimizing both the ECM and culture media formulations using a high-throughput microfluidic device. In several similar cases, optimized culture conditions successfully improved the growth of cancer organoids and vascularization of kidney organoids within microfluidic arrays.[68,75] In addition, microfluidic devices can be easily integrated with high-throughput analysis equipment to screen generated organoids according to certain standards. Uranga et al. developed a miniaturized inverted microscope that matched a high-throughput microfluidic platform. Such a platform can be used for effective imaging over time of the growth of tumor organoids in a controlled environment, as well as to record and screen the growth status of organoids. Microfluidic arrays with high organoid yields are promising platforms for organoid expansion and screening, which are crucial in the fields of drug discovery and regenerative medicine.

2.5. Mimicking Tissue Microenvironment in Organoid Culture

Controlling the microenvironment in vitro is essential for constructing more physiologically relevant organoids. Microfluidic chips provide an effective means to precisely manipulate various parameters such as chemical gradients, oxygen levels, and mechanical forces, which are crucial for the growth and differentiation of organoids. In this section, we discuss representative strategies compatible with microfluidics for controlling organoid culture microenvironments in terms of biochemical conditions and biophysical issues.[86]

2.5.1. Biochemical Conditions

Culturing stem cells in a 3D environment is a common strategy for constructing organoid models. The ECM is a complex macromolecular network with biochemical functions. Its composition and properties vary considerably, affecting the tissue-specific differentiation of stem cells. A representative example is a microfluidic chip coated with Matrigel overnight to improve the organoid adhesion. Moreover, it is essential to consider the effects of biochemical conditions in the ECM on the development and maturation of organoids when constructing 3D organoid models using microfluidics. Lee et al. utilized a kidney organoid-on-a-chip platform to study the effects of the biochemical factors in Matrigel on organoid vascularization.[75] With optimized preconditioning, the most significant specific area of renal markers was observed in an organoid-on-a-chip system coated with a 1.5% matrix containing 100 ng mL^{-1} VEGF. Even under static conditions, the addition of VEGF improved the expression of PECAM1, demonstrating that the VEGF-containing ECM may be a critical factor in the differentiation of hiPSCs into renal organoids and in their angiogenesis.

Recent studies have also shown enhanced cellular responses to extrinsically secreted factors in restricted microfluidic systems. Notably, these studies have provided quantitative comparisons of protein abundance between microfluidic and conventional culture conditions to describe the cellular secretome during the early stages of differentiation using high-throughput proteomic analyses. Because the accumulation of endogenous factors improves in a shorter period in a confined microfluidic environment, hepatocyte-like cells show higher cytochrome activity and albumin secretion.[53] In addition, it has been reported that organoid development requires much detail in morphogenetic signaling pathways to control stem cells' growth and cell-fate specification. Demers et al. harnessed microfluidic chips to address these challenges.[65] In this microfluidic system, a pair of microchannels was developed to generate stable morphogen gradients via Fickian diffusion across embryonic stem cells

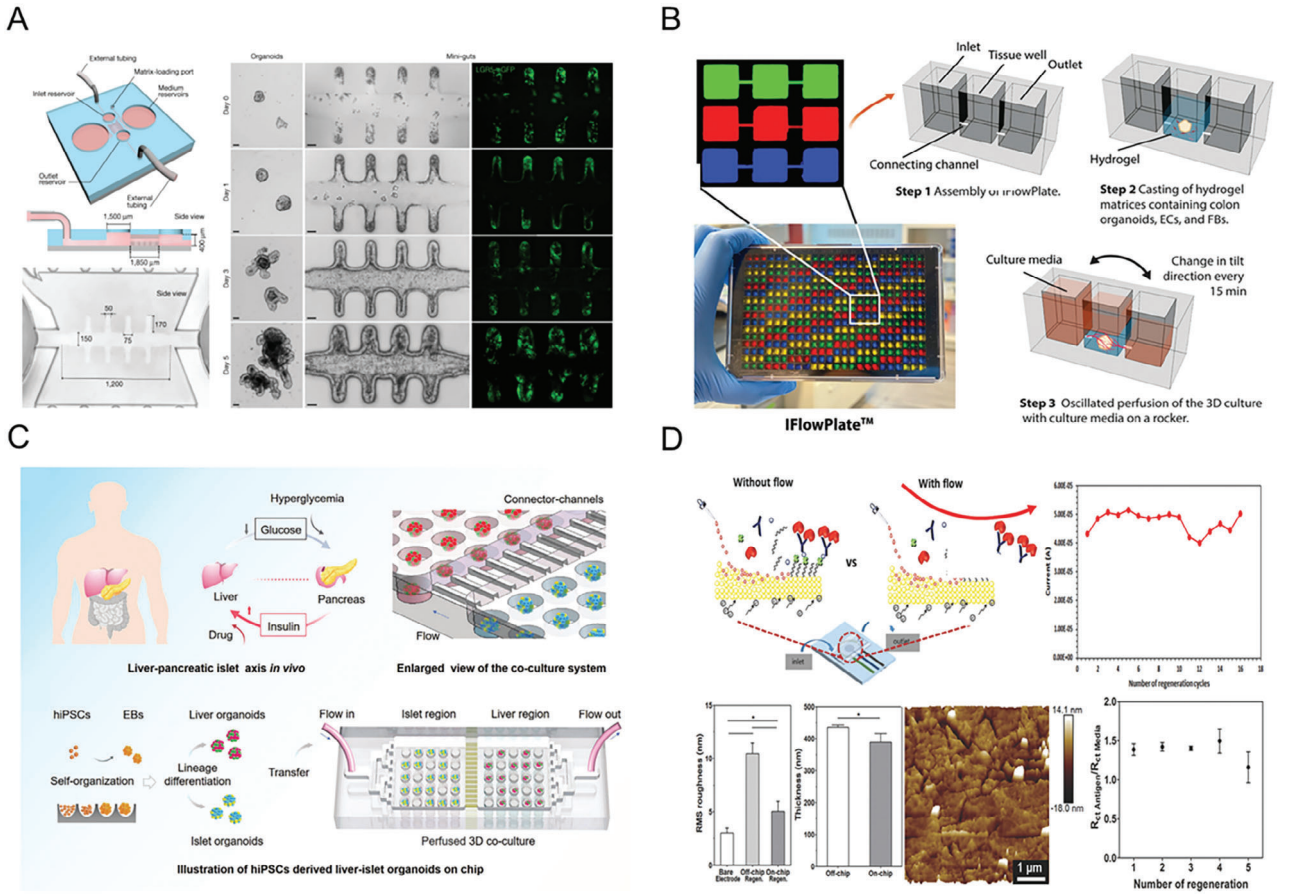

Figure 4. Representative examples of Microfluidic chips for long-term and functional organoid culture. A) Microfluidic chips to control the biophysical issues of organoid system. In this work, the authors built some microcavities on a microfluidic chip by laser ablation to mimic the geometry of anatomical crypts in the mouse small intestine. Reproduced with permission.[87] Copyright 2020, Springer Nature. B) Microfluidic chips to improve organoid vascularization. In this work, a customized microfluidic platform comprised of 384-well plate was reported for growing vascularized colon organoids in up to 128 independent units. The perfusable vascular bed was generated with human primary endothelial cells mixed and fibroblasts under fluid shear forces. Reproduced with permission.[85] Copyright 2020, Wiley-VCH. C) Microfluidic chips to replicate multi-organ interaction using organoids. In this work, the authors developed a dynamic culture chip to realize the hepato-islet interaction using organoids derived from hiPSCs. Compared to single organoid system, this chip is beneficial to promote the enhancement of the secretion function of liver and islet organoids and improve the expression of organ-specific functional genes and proteins. Reproduced with permission.[71] Copyright 2022, Wiley-VCH. D) Microfluidic chips for biosensing of organoids. In this work, electrochemical biosensors for automatically monitoring the metabolic activity of organoids were integrated into a human liver organoid-on-a-chip platform. When monitoring hepatotoxicity, diverse liver biomarkers such as GST-α and human albumin can be detected without labeling in this chip by the EIS. Reproduced with permission.[98] Copyright 2017, Wiley-VCH.

(ESCs)-laden hydrogels to model the neural tube development microenvironment in vitro.

2.5.2. Biophysical Issues

In addition to biochemical conditions, biophysical cues are essential factors for the construction of physiologically relevant organoid models. Therefore, mechanical forces represent the biophysical conditions that maintain the normal function of tissues or organs. Cells face many mechanical stimuli that regulate their behavior in vivo. Peristalsis stimulates the proliferation and differentiation of enterocytes, increases cell–ECM interactions, and influences cell migration during inflammation and wound healing. In recent years, combining microfluidic chip systems and the culture of intestinal organoids has created an opportunity to replicate the intestinal peristaltic microenvironment to bridge the large gap between the organoid model and colon tissue in vivo. Fang et al. proposed a microfluidic chip with a surrounding pressure channel and hundreds of lateral microwells to simulate peristalsis for culturing human colon tumor organoids, as shown in **Figure** 4A.[46] These biomimetic designs allow colon organoids to grow under controllable peristaltic amplitudes and rhythms. Interestingly, organoids showed reduced uptake of ellipticin-loaded polymeric micelles during peristalsis in vitro when evaluating the efficiency of nanomedicine, indicating that mechanical stimuli are essential for constructing more reliable organoid models for drug screening. Similarly, Lee et al. reported an innovative microfluidic chip containing luminal flow and peristaltic-like motility that rhythmically introduced stretching and contraction to human gastric organoids derived from hiPSCs via a peristaltic pump.[54] Utilizing this platform, the potential to deliver various

nutrients or pharmacological compounds into the gastric lumen may be further developed for long-term studies of human gastric physiology, drug screening, and disease modeling, among other possibilities in vitro.

The geometrical topography of microfluidic chips is another necessary biophysical condition for constructing organoids, because of the complex and hierarchical structures of their tissue or organ counterparts in vivo. For example, Nikolaev et al. built microcavities on a microfluidic chip using laser ablation to mimic the geometry of anatomical crypts in the mouse small intestine.[87] In this device, intestinal stem cells can form tubular epithelial cells with a spatial arrangement of crypt- and villi-like features similar to those in vivo. In conclusion, these studies demonstrated how microfluidic chips and organoids can be combined to achieve in vitro modeling of higher-order organoids with dynamic and physiologically relevant properties, which would not be possible using either technique alone.

2.6. Vascularization of Organoids

The absence of vascular networks restricts the exchange of oxygen, nutrients, and metabolic waste within organoids. As organoid spheroids grow to a dimensional scale of ≈150 µm, cell proliferation ceases, and necrosis quickly occurs in their core region.[88] It is reported that the various physical cues such as fluid shear forces have been reported to play crucial roles in influencing endothelial cell junctions and facilitating their assembly into vascular networks. Therefore, there is no doubt that microfluidic chips have the advantage of precise control of fluid flow and shear stress, which can also provide great opportunities in enhancing vascularization and improving organoid function.[51,52,57,67,75,84,85,89,90] For instance, Rajasekar et al. reported a customized microfluidic platform comprised of 384-well plate for growing vascularized colon organoids in up to 128 independent units, termed IFlowPlate (Figure 4B).[85] Unlike traditional closed microfluidic devices, the IFlowPlate's open-hole design enables easy retrieval of culture models for histochemical analysis and even in vivo transplantation. During the culture period, a programmable rocker can drive the medium from the inlet to the intermediate and outlet channels of the device, generating fluid shear forces. Using this plate, human primary endothelial cells and fibroblasts can self-assemble into a perfusable vascular bed within 3 days. Similarly, when hiPSCs and human endothelial cells were co-cultured in millifluidic chips, massive blood vessel formation within kidney organoids was observed under fluid stimulation.[52] In fact, further research into the optimization of microfluidic chip design and culture conditions will undoubtedly lead to even greater advancements in the field of organoid vasculature engineering.

2.7. Multi-Organoids Interactions

Although organoids have shown superior vitality and function as in vitro models, the complexity and interactions between different organs in the construction of organoid systems remain unknown. In recent years, multi-organoids on chips have been extensively developed.[71,91–97] Through some micro-engineering designs, such microfluidic chips can co-culture different types of organoids to achieve more detailed biological simulations. Rajan et al. established an integrated multiorganoid system with up to six tissue types (liver, lung, heart, brain, endothelium, and testis) to evaluate functional drug responses.[91] The key aim of this study was to simulate the effects of the products metabolized by the liver on other organs in vitro. Furthermore, the functionality of this organoid-integrated system was tested separately using ifosamide and capecitabine drugs. In the presence of liver organoids, each drug was metabolized into products with downstream neurotoxicity for other organoids (lung, cardiac, or brain tissue, depending on the specific drugs), while removing the liver from the system did not result in significant toxicity within any tissue type. Tao et al. constructed a hepato-islet organoid interaction system derived from hiPSCs (Figure 4C).[71] The dynamic culture and interaction of liver and islet organoids were studied on a microarray chip designed by partitioning, and organoid function was maintained for up to 1 month. Using this model is beneficial for enhancing the secretory function of liver and islet organoids and improving the expression of organ-specific functional genes and proteins. Furthermore, adding metformin, a commonly used hypoglycemic drug, to the system significantly improved the liver and islet pathological damage caused by high glucose conditions. This indicates the feasibility and application prospects of this new organoid interaction chip system for disease simulation and drug evaluation. To date, the advantages of multi-tissue organ-on-a-chip compared to single organoids have been illustrated, but using these models to mimic all the physiological characteristics of the human body remains a huge challenge.

2.8. Integrated Detection of Organoids with Electrochemical and Imaging Techniques

In addition to the rational design of microfluidic device structures, microfluidic systems can be integrated with electrochemical sensing, imaging, and other technologies to monitor organoids in real-time. The ability to accurately and continuously monitor biomarkers in organoid culture systems is critical for evaluating the cellular state or biological effects of drugs on organoids over extended periods. Microfluidic systems have great potential for integrating various sensors to convert biochemical signals into electrical signals and evaluate relevant functional molecules secreted by cells during organoid culture.[69,98–100] In such systems, oxygen, creatine kinase, and cell metabolites can be detected in situ by setting microelectrode with or without premodification into microfluidic devices. A good example is shown in Figure 4D, in which a human liver organoid-on-a-chip platform is integrated with electrochemical biosensors to automatically monitor the metabolic activity of this biomimetic model.[98] When monitoring hepatotoxicity, diverse liver biomarkers such as glutathione-S-transferase-alpha (GST-α) and human albumin can be detected without labeling in this chip by electrochemical impedance spectroscopy (EIS). Notably, the robust sensing platform provides a means to replace the enzyme-linked immunosorbent assay for the convenient monitoring of the dynamic behavior of cells in organoids. This provides great opportunities for the use of organoids to screen drug efficacy,

toxicity, and pharmacokinetics. Nashimoto et al. developed an open-top microfluidic device with sensor capabilities to monitor oxygen metabolism in patient-derived cancer organoids.[100] The organoids were cultured on a membrane with micropores, under which there were perfusable microchannels. To detect the oxygen metabolism in the organoids, an external electrode was inserted into the organoids during culture. This study provides a simple platform for monitoring the metabolic levels of 3D cultured cells integrated with a perfusable vascular network.

To increase the efficiency of signal acquisition or detect large volumes of organoids, microelectrode arrays are combined with microfluidic systems.[101,102] For example, Liu et al. developed a taste bud organoid-based biosensor for the research of taste sensation.[102] In this study, large taste bud organoids derived from newborn mice were cultured on the surface of a 64-channel microelectrode array chip, which was used to simultaneously record multiple neuronal firing activities from the organoids under different taste stimuli. Such a design would help reveal the role of taste buds in taste sensing. Imaging is another necessary method by which the state of organoid models can be assessed through direct observation.[103–105] These visualized microfluidic systems are powerful tools for investigating and recording organoid morphogenesis and specific physiological processes, such as calcium transients. In a representative case, Khan et al. described a low-cost and 3D-printed microfluidic bioreactor that can simultaneously support organoid imaging and cultivation in a single chip.[103] Such a platform allows for the in situ tracking of the brain organoid self-assembly process, which is highly beneficial for understanding the dynamics of organoid development. Because the combination of smart microfluidic systems and organoids is in its infancy, we envision that more detection technologies for organoids, such as patch clamps[106] and nanoelectronics,[107] can be fabricated in microfluidic devices to increase the feasibility of better monitoring of cultured organoids.

3. Microfluidic Fabrication of Hydrogels in Organoids Culture

The components and structural properties of biomaterials are essential for guiding cell proliferation, migration, and differentiation of organoids, thus affecting their formation and function. In recent years, microfluidic platforms have been regarded as reactors for the continuous synthesis of various biomaterials with defined compositions and morphologies. These materials also exhibit good biocompatibility or specific physical and chemical properties and are widely used in biomedical fields, such as tissue engineering and drug delivery. Diverse 3D scaffolds produced using microfluidic technology, including microgels and microfibers, have been incorporated into organoid research. In the following subsections, we introduce these points.

3.1. Microgels Generated by Droplet Microfluidics

Microgels have emerged as reliable 3D culture scaffolds and transplanted cellular carriers in biomedicine, owing to their proper permeability, flexible self-assembly, and ability to scale-up production. Most microgel applications are strongly influenced by the form, size, and size distribution of microgels. Droplet microfluidic technology is widely used to manufacture highly monodisperse microgel particles for various applications, including organoid engineering.[108–115] For instance, Fang et al. reported a nonadhesive alginate microgel produced using a droplet microfluidic device for mammary tumor organoid culture.[108] As shown in **Figure 5A**, the established organoids displayed luminal- and solid-like structures and possessed high similarity to primary tumor tissues in their cellular lineages and phenotypes. Furthermore, the lumen pressure of the organoids increased with lumen growth and ultimately reached 2 kPa after 2 weeks of culture. Zhang et al. used droplet microfluidics to generate complete organoid precursors from cell-laden Matrigel.[109] This prevents organoids from growing from small units and reliably fulfills the goal of obtaining large organoids in one step. Compared with the production of large organoid models by traditional culture technology (i.e., using single cells or cell clusters in Matrigel to produce organoids), the droplet template method can make organoids with a diameter of 500 μm and above in only 1 week.

By exploiting the flexibility of droplet microfluidics, microgels with hybrid components and advanced morphologies can be produced to promote the development of organoids. For example, Liu et al. developed an all-in-water droplet microfluidic platform for the controllable fabrication of hybrid hydrogel capsules that allowed massive formation and culture of uniform islet and liver organoids derived from hiPSCs.[110,111] In this study, binary capsules were fabricated by the interfacial complexation of Na-alginate and chitosan. The encapsulated islet and liver organoids with consistent sizes consisted of organ-specific subtypes of cells and showed their respective secretory functions, including glucose-stimulated insulin secretion and albumin secretion, which may offer a robust platform for accelerating organoid research and translational applications. In conclusion, these droplet-based scaffolds can be used as templates to control the size and reproducibility of organoids and provide an ECM-like niche for organoid engineering.

3.2. Fibers Fabricated by Microfluidic Spinning

Fibers usually refer to materials with a high length-to-diameter ratio and flexibility and strength. Recently, microfluidic spinning has allowed the preparation of fibers with fine control of shape, size, chemical anisotropy, and biological activity and has become a very promising strategy for the synthesis of micro- and nanoscale polymer materials. In addition, combining hydrogels with microfiber-based scaffolds can create flexible and physiologically relevant tissue models that have attracted extensive attention for various biomedical applications, including 3D cell culture, tissue modeling, and clinical therapy. With conveniently observable and flexible tissue manipulations and the scalable characteristics of these microfibers, recent studies have demonstrated that some organoids encapsulated in microfibers enable the construction of ideal biomimetic organ models in vitro. In an early study, Zhu et al. generated massive brain organoids from hiPSCs within adjustable calcium alginate hollow fibers by combining a multilayer coaxial laminar flow in a microfluidic system.[116] In particular, these brain organoids show many

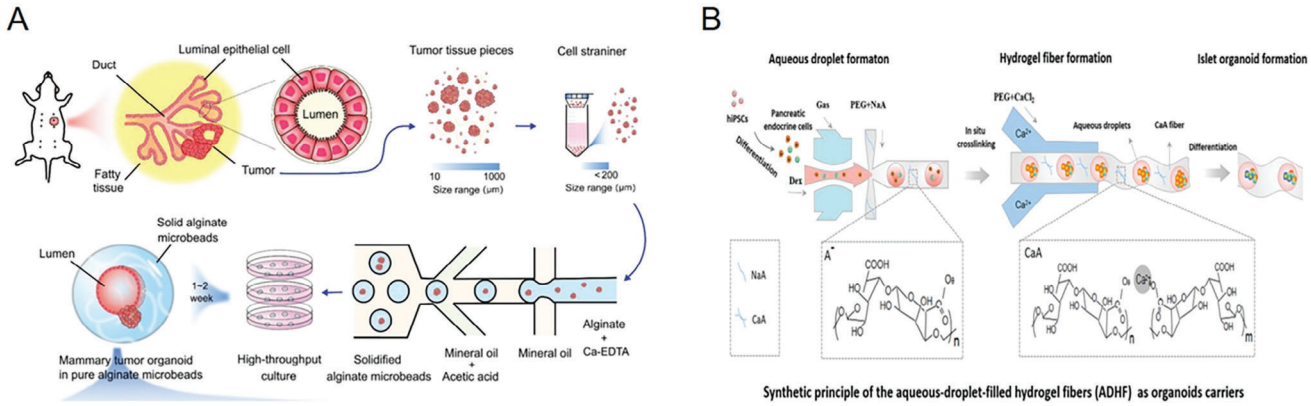

Figure 5. Representative examples of scaffolds generated in microfluidic reactors for organoid engineering. A) Alginate microgels generated by droplet microfluidics for organoid encapsulation and 3D culture. In this work, non-adhesive alginate microgels were produced by the droplet microfluidic device for mammary tumor organoids culture, which displayed luminal- and solid-like structures and possessed a high similarity to the fresh tumor in their cellular lineages and phenotypes. Reproduced with permission.[108] Copyright 2021, Wiley-VCH. B) Hydrogel fibers generated by droplet microfluidic spinning for organoid encapsulation and 3D culture. In this work, the authors designed a novel aqueous microfluidic platform integrated with a pump valve cycle system that allows the ADHFs to fabricate functional human islet organoids in situ. Reproduced with permission.[117] Copyright 2021, American Chemical Society.

key features of human brain organogenesis, such as cell-type heterogeneity, a polarized neuroepithelium, and discrete brain regions, similar to early brain development.

Recently, with further advancements in microfluidic technology, various morphological characteristics of microfibers such as tubular, helical, grooved, and knotted have emerged. Among these, knotted hydrogel fibers are beneficial for accelerating cell self-assembly because of the existence of internal cavities, which also present additional potential for the construction of controllable organoid spheroids. To verify this hypothesis, Wang et al. designed a novel aqueous microfluidic platform integrated with a pump–valve cycle system that allowed aqueous droplet-filled hydrogel fibers (ADHFs) to fabricate functional human islet organoids, as shown in Figure 5B.[117] After 7 days of culture, the islet organoids within such fibers exhibited good cellular viability and high sensitivity to glucose in stimulating insulin secretion and the upregulation of islet-specific genes. These examples have showcased the potential of various fibers fabricated by microfluidic spinning to place cells in a more physiological microenvironment, thereby promoting organoid maturation. However, the selection of microfluidic spinning materials often requires consideration of the mechanical strength, biocompatibility, and adaptability of the cross-linking methods, which cause many hydrogel materials to encounter bottlenecks in the construction of cellular microfiber carriers. For example, although the alginate hydrogel is successfully compatible with microfluidic spinning technology, some inherent shortcomings, such as low mechanical strength and lack of cell adhesion sites, cannot be ignored. Improving the material properties of alginate hydrogels by constructing a double network structure and performing RGD sequence modification may help microfluidic spinning play a stronger role in large-scale and biomimetic organoid cultures.[118,119]

3.3. Complex Hydrogel Scaffolds Constructed by Microfluidic Bioprinting

Bioprinting technology can be used to design and produce complex scaffolds filled with cells layer-by-layer. Recently, the combination of microfluidic systems with traditional extrusion-based bioprinting has enabled a more flexible adjustment of the structural and compositional characteristics of tissue/organ constructs during the printing process. This offers the possibility of creating next-generation engineered tissue/organ constructs with complex and ordered structures, coinciding with the concept of organoid development.[120] For instance, Serex et al. proposed a microfluidics-based bioprinting technology capable of tuning the concentration of printed cells in real time.[121] Using this method, fibroblasts can be distributed and concentrated to a concentration of up to 10 million cells mL^{-1}, enabling high cell concentration bioprinting. In addition, because cell–cell interactions are essential for organoid formation, the ability to concentrate cells is crucial for standardizing the process of organoid generation and obtaining more stable and reproducible results. For verification, urothelial cells isolated from mice were deposited in culture dishes at densities of 0.125, 0.5, and 2 million cells mL^{-1}, respectively. Interestingly, organoids with a central lumen structure were found only at a density of 0.5 million cells mL^{-1}. This emphasizes that the optimal cell concentration for organogenesis lies in the middle range, where organoids can form quickly without degrading the surrounding matrix. Overall, the manufacture of organoids involves combining many variables in a complex culture regimen, and this complexity often leads to dramatic changes in the shape of the organoids or even complete failure. Bioprinting combined with microfluidics can greatly simplify and determine the time and resources required to produce optimal conditions for a given type of organoid.

4. Organoids Utilized in Biomedical Applications

The incorporation of organoids into microfluidic systems has contributed to significant advancements in tissue engineering and personalized medicine. By offering precise control over fluid distribution and nutrient supply, microfluidic systems allow for a more accurate mimicking of the dynamic microenvironments of various organs in vivo. Compared to common animal models or static cell cultures, organoids grown in microfluidic systems are attractive organotypic models for biomedical and basic medical research. In addition, the utilization of microfluidic systems enables better control over the high variability in organoid size, architecture, cell phenotype, and composition, thereby further expanding the application of biomimetic organoids in a wide range of fields, such as organ development, disease research, drug testing, regenerative medicine, and tumor immunotherapy.

4.1. Organ Development

Organoids can reproduce several major events of organ development in vivo, and hold great potential for exploring key physiological processes in vitro. Traditional organoid systems cannot support long-term culture and controllable manipulation of the tissue environment, which critically limits systematic studies on developmental biology. In contrast, microfluidics has been demonstrated to manipulate tiny fluids that produce a variety of autocrine or paracrine cytokines to form different niche properties by controlling the shear stress, interstitial fluids, and cell density. These niche properties potentially affect the morphology, proliferation, and metabolic activity of organoids. For example, Michielin et al. presented a microfluidic device with a confined environment to investigate the role of the self-organizing ECM in hepatic commitment and organoid formation from hiPSCs (**Figure 6A**).[53] The authors found that liver progenitor cells exposed to exogenous ECM stimulation showed a significantly higher potential to form liver organoids that could rapidly expand over several generations and further differentiate into functional liver cells. This study also indicated that a controllable balance between soluble endogenous and exogenous factors within microfluidics significantly affects the maintenance of pluripotency, germ layer regulation, and liver differentiation of hiPSCs. In several similar cases, exogenous ECM combined with continuous shear stress promoted the development and maturation of kidney and brain organoids in microfluidic systems, replicating the key progress of the electrophysiological function of the brain and vascularization of the kidney.[75,92]

Microfluidic systems equipped with biosensing and imaging units can be used to monitor organoid formation and morphogenesis. Karzbrun et al. presented a microfabricated compartment within a microfluidic chip to support the culture of brain organoids and allowed for in situ imaging over a timescale of weeks.[104] With this chip, the authors investigated the appearance of the surface wrinkles implicated in neurodevelopmental disorders during the development of brain organoids in vitro. They found that convolutions emerged at a critical cell density and maximal nuclear strain, at which the wrinkling wavelength exhibited linear scaling with tissue thickness and was consistent with balanced bending and stretching energies. In addition, microfabricated chips can be endowed with biomimetic geometries to guide the morphogenesis of organoids, for example, by constructing intestinal crypts and centimeter-long motor nerves in vitro.[87,122] In summary, microfluidics have improved the feasibility of precisely regulating the microenvironment of organoid development and recording dynamic processes in situ.

4.2. Disease Modeling

In addition to physiological processes, organoids with high relevance to human organs can be utilized to reproduce pathological phenomena in vitro to reveal underlying mechanisms or explore novel therapeutics. Generally, the construction of disease models using organoids involves two strategies: generation of organoids with healthy cells and induction by biochemical stimuli,[66,71,79,80,93,101] and production of organoids with patient-derived cells.[55,56,60] A typical case of the former is displayed in Figure 6B, in which the authors proposed a microfluidic strategy to build a human nonalcoholic fatty liver disease (NAFLD) model with hiPSC-derived liver organoids.[79] In this study, hiPSC-derived hepatic progenitors were seeded onto a micropillar-integrated chip to form spheroids before generating liver organoids upon subsequent chemical induction. The NAFLD model was established by exposing organoids to free fatty acids in perfused 3D cultures. Finally, the proposed liver organoids showed the pathological features of NAFLD, such as upregulated expression of lipid metabolism-associated genes and production of ROS production. In another case, Ao et al. developed an innovative organoid microphysiological analysis platform that can realize the generation of organoids and their interaction with immune cells, and reproduce the key characteristics and processes of neuroimmune interaction in the elderly brain with a damaged brain barrier.[93]

Patient-derived organoids are an a priori option in personalized medicine due to the preservation of patient-specific genetic backgrounds. In a typical case, Hiratsuka et al. built a millifluidic device to investigate disease pathology and construct therapies for autosomal recessive polycystic kidney disease (ARPKD).[55] The ARPKD organoids were generated from patient-derived induced pluripotent stem cells (iPSCs), which were cultured in perfusable chips and static wells, respectively. The authors found that PKHD1 mutants within organoids could generate cystogenesis only in perfusable chips that produced fluids similar to the native tissue microenvironment in patients with ARPKD. Moreover, mechanosensing molecules, including RAC1 and FOS, were identified as potential therapeutic targets, which were validated using kidney samples from patients. Similar attempts have been made to construct other disease models using patient-derived organoids, such as Crohn's disease, ulcerative colitis, and retinitis pigmentosa,[56,60] indicating the universality of such a strategy. In addition, various tumor models have been established in vitro using patient biopsies to expand and form organoids in microfluidic systems. The types of tumors involving the breast, pancreas, and colon[57,61,67,74,82,83,94] mainly focus on relevant drug screening, which will be discussed in the next section. Understanding the interaction between the tumor microenvironment (TME) and immune system is also a key step in developing novel and highly

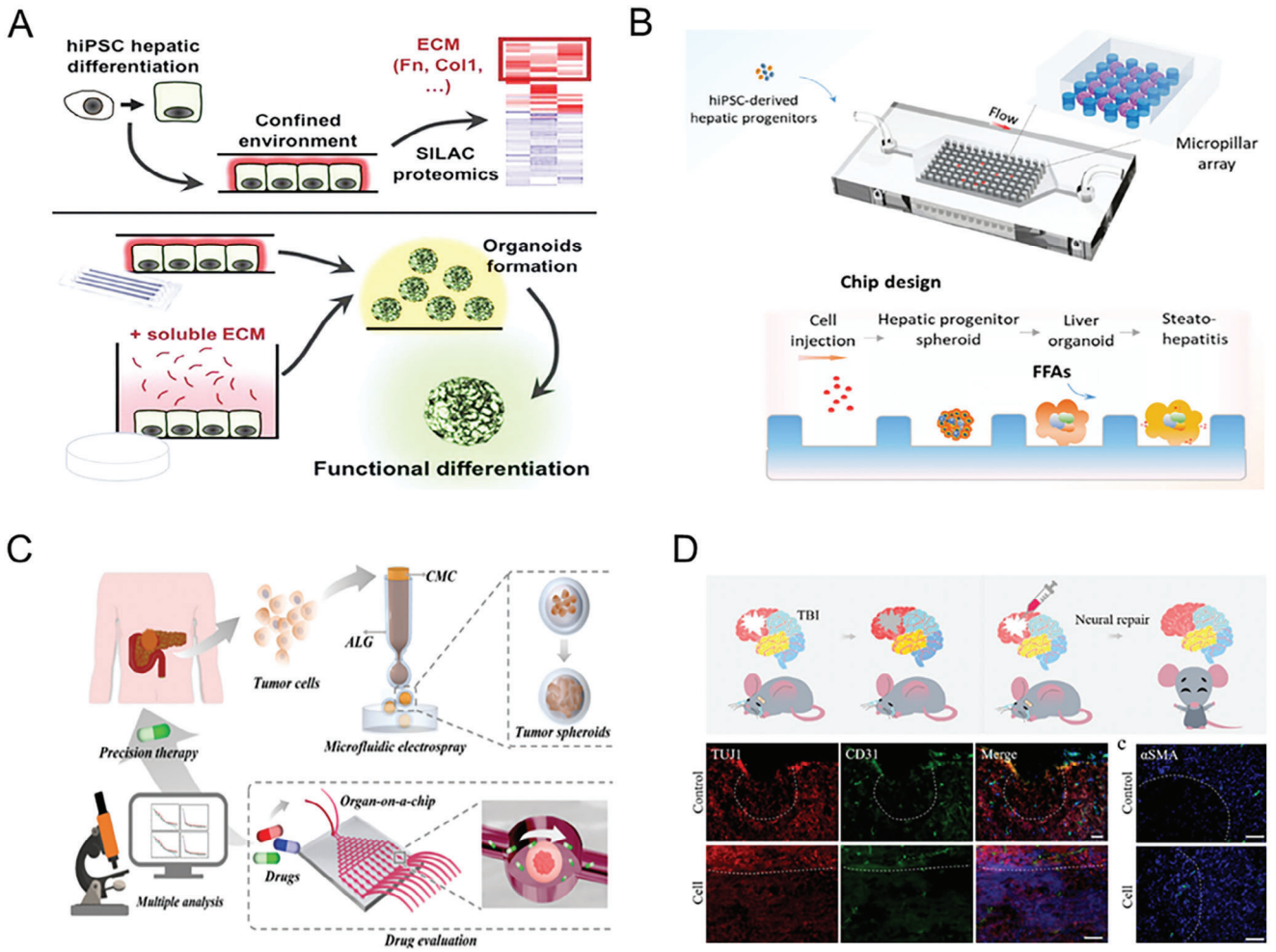

Figure 6. Representative applications of integrating organoids with microfluidic systems. A) Microfluidic device with confined environment was used to investigate the role of self-organizing ECM in hepatic commitment and organoid formation from hiPSCs. When stimulated by exogenous ECM, hepatic progenitor cells showed significantly higher potential to form rapidly expandable liver organoids. Reproduced with permission.[53] Copyright 2020, Elsevier. B) hiPSC-derived hepatic progenitors were seeded in a micropillar-integrated chip to generate liver organoid. Then, these perfused 3D cultures can be investigated the human nonalcoholic fatty liver disease by exposure to free fatty acids. Reproduced with permission.[79] Copyright 2020, American Chemical Society. C) Pancreatic tumor spheroids encapsulated in alginate microcapsules were transferred to a microfluidic chip with eight stages of branched channels for dynamic and high-throughput screening of different chemotherapy regimens. Based on this approach, the drug sensitivity of 3 different patient-derived tumor spheroids was explored. Reproduced with permission.[112] Copyright 2023, Wiley-VCH. D) By utilizing a microfluidic electrospray platform, hiPSC-derived neural cells were encapsulated in hydrogel microcapsules to form pre-vascularized brain organoids. After injecting this neural model into rats, it was found that nerve regeneration was promoted after traumatic brain injury. Reproduced with permission.[113] Copyright 2021, Elsevier.

effective cancer immunotherapies. Microfluidic tumor organoid-on-a-chip platforms are innovative tools with several unique advantages, such as low reagent consumption, versatile structures and function designs, dynamic and precise fluid control, and the ability to facilitate 3D cell co-culture. These platforms are capable of recapitulating the key factors of the TME and immune context. They have been recognized as reliable tools for studying how tumors regulate their TME to counteract antitumor immunity, and the mechanisms underlying tumor resistance to immunotherapy.[123] Briefly, burgeoning organoid models have provided superior candidates for exploring new insights into various diseases and developing appropriate therapeutics.

4.3. Drug Testing

In drug development, especially for rare diseases or diseases lacking large-scale clinical trials, organoids are excellent models for drug toxicity prediction and new drug screening, providing sufficient resources for deep sequencing, functional testing, mutation sites, and phenotype analysis. With the inclusion of microfluidic technology, these complex microphysiological systems reconstruct the in vivo cross-talk between different organs, providing powerful models of human physiology for drug research. For example, Skardal et al. described a three-organ model comprising bioengineered liver, heart, and lung organoids using a

closed microfluidic perfusion system.[95] In this study, the interorgan responses to drug administration were explored. Notably, in a system without liver organoids, propranolol prevented adrenaline-induced increases in the heart rate. However, when liver organoids were seeded into the system, propranolol was metabolically inactivated and the beat rates of cardiac organoids increased after epinephrine administration, confirming the necessity of liver constructs for drug testing. These results have meaningful implications for improving organoid model systems to predict the processes of drugs, chemicals, or biologics more accurately in the human body.

In addition, high throughput, high analysis efficiency, and low economic and time costs are major challenges for pharmaceutical analysis. The high throughput, high analytical efficiency, low reagent consumption, and automated capabilities of the microfluidic platform further raise the possibility of using organoids for high-throughput drug screening.[34,46,74,77,95,97,108] In a recent case, Song et al. described an efficient tumor organoid chemotherapeutic drug evaluation platform by combining the microfluidic chip with eight stages of branched channels (Figure 6C).[112] Primary human pancreatic cancer cells were encapsulated in alginate microcapsules to form 3D tumor spheroids of uniform size. The microcapsules were then transferred to the culture chamber of the chip. The dendritic branch channel of the chip can be used as a concentration gradient generator to establish different drug concentration gradients in the culture chamber for the dynamic and high-throughput screening of different chemotherapy regimens. Based on this approach, they compared the drug sensitivities of three different patient-derived tumor spheroids, and the results were consistent with the clinical data of the corresponding patient. These studies show that microfluidic technology provides strong support for highly sensitive and specific drug analysis.

4.4. Regenerative Medicine

Regenerative medicine is an important application in the field of organoids that focuses on reproducing functional tissues and organs in vitro and transplanting them into the human body to repair or replace dysfunctional tissues. Organoids can provide infinite donor- and patient-derived tissues for clinical applications. Although the cell sources of organoids, including stem cells and adult cells, have already represented a substitute for cadaver and organ transplantation, cell-based therapy has inherent problems, mainly due to the short retention time and weak mechanical properties of the isolated cells.[124] Compared to discrete cells, cell spheroids show enhanced paracrine effects, higher expression of anti-apoptotic proteins, and stronger cell-cell/cell-ECM interactions, which are more promising for transplantation therapy.[125,126] Massive stem cell spheroids within microgels can be produced using droplet microfluidics, owing to their high-throughput and controllable properties for regenerative medicine.[127–129] For example, Zhao et al. developed a microfluidics-assisted strategy to regenerate bone in vivo using bone marrow-derived mesenchymal stem cells (BMSCs).[127] In this study, droplet microfluidics were used to co-encapsulate BMSCs and growth factors into GelMA microgels transplanted into rabbit femoral defects. The results showed that the cell-loaded microgels effectively improved new bone formation after transplantation for 4 weeks, as confirmed by the bone volumes and extent of osteoid formation.

Organoids that can reproduce the key structural and functional properties of living organs are better candidates for clinical applications than simplified cell spheroids. In particular, prevascularized organoids exhibit unique advantages in connecting to host blood vessels to allow for the survival and function of grafts. In a typical case, a proof-of-concept experiment was performed in which brain organoids were transplanted into rats with traumatic brain injury, as shown in Figure 6D.[113] In this study, the authors used a microfluidic electrospray platform to encapsulate hiPSC-derived neural cells in hydrogel microcapsules cocultured with endothelial cells and fibroblasts to form complex vascular networks. Then, the pre-vascularized brain organoids were transplanted into traumatic brain injury rats for 2 weeks, after which the neural regeneration within rat brain was confirmed by expression of CD31+, α-SMA+, and TUJ1+ signals. In summary, functional organoids have opened new possibilities for repairing or replacing the dysfunctional tissues of patients, which call for further verification before clinical therapeutics.

5. Summary and Outlook

Organoids have already been established as promising near-physiological 3D models with an astonishing resemblance to native organs, both in terms of tissue architecture and key biofunctions. The integration of microfluidic technologies into the field of organoids has further accelerated the progress of organoid engineering in terms of high-throughput manipulation, vascularization, and controllable microenvironments. In particular, high-throughput formation and expansion of organoids can be achieved using microfluidic reactors. Dynamic microfluidic culture platforms are promising for the long-term maintenance of organoids and partially realizing vascularization progress. In addition, the controllable microenvironment in microfluidic devices exhibits positive effects on improving organoid complexity and functionalities (**Table 1**). Although the combination of organoids and microfluidics has displayed significant advantages in the fields of organ development, disease studies, drug screening, and regenerative medicine, achieving high-fidelity tissues that are analogous in vitro remains a major challenge.

First, the controllability of microfluidic devices in the tissue microenvironment is insufficient for reproducing the developmental process of in vivo organs using organoids. More complex factors such as stimuli-responsive biomaterials, continuous fluid supply, gradients of biochemical factors, and mechanical stimulation should be merged into a single system to increase the biomimetic properties of the microenvironment.

With the rise of microfluidic technology used in research on organoid chips, selecting microfluidic materials for processing often requires compromises between the ease of model production and device performance. PDMS is the most commonly used elastomer in microfluidic devices because of its low cost, easy-to-use soft lithography processing, optical transparency for cell imaging and evaluation, high elasticity, and good oxygen permeability for long-term cell cultures in enclosed microfluidic channels or chambers. However, some difficult-to-ignore shortcomings, especially in absorbing small hydrophobic molecules and

Table 1. Summary of the microfluidic technologies used in organoid systems and the typical applications.

Microfluidic systems	Major properties	Cell sources	Organoid types	Applications
Microwell arrays	• Massive generation of organoids • Improving the uniformity of organoids in size and morphology • Capable of integrating with other microfluidic technologies	hiPSCs	• Brain[76] • Intestine[34] • Kidney[75] • Pancreas[33,70]	• Reducing glycolytic stress in cerebral organoids[76] • Anti-tumor drug screening[34] • Nephrotoxic drug testing[75] • Budling pancreatic duct-like models[70] • Modeling the development of islet[33]
		hESCs	• Retina[72]	N.A.
		Human tumor biopsies	• Intestine[46,77] • Pancreas[74] • Lung[73]	• Anti-tumor drug screening[46,74,77] • Testing anti-tumor drug sensitivity[73]
		Mouse crypts	• Intestine[34]	• Anti-tumor drug screening[34]
Micropillar arrays	• Massive generation of organoids • Improving the uniformity of organoids in size and morphology • Improving nutrient and waste exchange • Capable of integrating with other microfluidic technologies	hiPSCs	• Brain[35,78,80] • Liver[36,79]	• Modeling the development of brain[35] • Modeling neurogenesis induced by Cadmium[78] • Studying effects of VPA exposure on the development of fetal brain[80] • Modeling the development of liver[36] • Modeling human nonalcoholic fatty liver disease[79]
Perfusable microchannel arrays	• Massive generation of organoids • Prolonging the organoid culture time • Providing controllable microenvironment • Realizing the vascularization of organoids	Primary human hepatocytes	• Liver[84]	• Investigating the interactions between organoids and vasculatures[84]
		Human tumor biopsies	• Intestine[85] • Pancreas[82,83] • Ovarian[85]	• Modeling colonic inflammation[85] • Studying the immune cell recruitment in tumor microenvironment[82] • Testing anti-tumor drugs[83] • Testing innovative anti-tumor drugs[68]
Droplet microfluidic platforms	• Massive generation of organoids • Improving the uniformity of organoids in size and morphology • Providing biomimetic ECM conditions • Great potential in organoid transplantation • Realizing the vascularization of organoids	hiPSCs	• Brain[113,114] • Liver[111] • Pancreas[110]	• Organoid transplantation for brain injury in rats[113] • Modeling early development of brain[114] • Modeling hepatic development[111] • Modeling the development of islet[110]
		Human tumor biopsies	• Intestine[109] • Intestine[115] • Pancreas[112]	• Anti-tumor drug testing[115] • Anti-tumor drug evaluation[112]
		Mouse tumor tissues	• Breast[108]	• Anti-tumor drug screening[108]
Microfluidic spinning platforms	• Massive generation of organoids • Providing biomimetic ECM conditions • Great potential in organoid transplantation • Improving flexibility in large-scale manipulation	hiPSCs	• Brain[116] • Pancreas[117]	• Modeling early development of brain[116] • Modeling the development of islet[117]
Microfluidic-assisted bioprinting	• Enabling high high-concentration stack of cells • Precisely controlling the organoid morphology • Improving the vascularization of organoids	Primary mouse tissues	• Bladder[121]	• Studying the importance of cell seeding concentration for organogenesis of organoids[121]

(*Continued*)

Table 1. (Continued)

Microfluidic systems	Major properties	Cell sources	Organoid types	Applications
Microfluidic chips with geometrical guidance	• Prolonging the organoid culture time • Providing controllable microenvironment • Providing biomimetic ECM conditions • Generating organoids with heterogeneous structures	hiPSCs	• Brain[122]	• Generating centimeter-long motor nerves[122]
		Human intestinal stem cells	• Intestine[87]	• Investigating intestinal infection caused by *Cryptosporidium parvum*[87]
Perfusable microfluidic devices with individual channels or chambers	• Prolonging the organoid culture time • Providing controllable microenvironment • Improving the vascularization of organoids • Providing mechanical stimulations	hiPSCs	• Intestine[58] • Kidney[52,55,59] • Liver[53] • Retina[56] • Stomach[54]	• Modeling the development of intestine[58] • Improving the maturation of organoids[52] • Modeling human autosomal recessive polycystic kidney disease[55] • Modeling basolateral drug transport and uptake in proximal tubules[59] • Providing insights into the hepatic development[53] • Exploring the retinitis pigmentosa with USH2A mutations
		Human tumor biopsies	• Pancreas[57]	• Recapitulating pancreatic tumor microenvironment[57]
Microfluidic chips with sandwiched porous membranes	• Prolonging the organoid culture time • Providing controllable microenvironment • Enabling co-culture of different cells • Great potential to build tissue barriers • Providing mechanical stimulations	hiPSCs	• Intestine[63] • Liver[47] • Heart[47] • Retina[64]	• Modeling the responses of intestines to exogenous stimuli, like IFN-γ[63] • Testing antidepressant drugs[47] • Modeling complex and multilayer retinal structures[64]
		Patient-derived biopsies	• Intestine[48,49,60,62]	• Modeling human Crohn's disease, ulcerative colitis, or colorectal cancer[60] • Modeling the development and geometry of small intestine[62] • Modeling the enteric coronavirus infection[48]
		Human tumor biopsies	• Pancreas[61]	• Testing anti-tumor drugs[61]
Perfusable microfluidic chips with parallel channels	• Prolonging the organoid culture time • Providing controllable microenvironment • Enabling co-culture of different cells • Great potential to generate factor gradients • Providing biomimetic ECM conditions • Realization the vascularization of organoids	hiPSCs	• Brain[31,32,51] • Vascular[51]	• Modeling early development of brain[31] • Studying effects of nicotine exposure on fetal brain development[32] • Exploring the interactions between brain and de novo-generated vasculatures[51]
		Human ASCs	• Liver[66]	• Modeling T-cell immunity against hepatitis C virus[66]
		Human tumor biopsies	• Breast[67]	• Anti-tumor drug testing[67]
		Mouse ESCs	• Brain[65]	• Modeling neural tube patterning[65]

(*Continued*)

high-throughput manufacturing of PDMS-based devices, have severely limited their application in studying cellular responses to drugs. Thermoplastic polymers, such as polystyrene, polylactic acid, and polymethylmethacrylate, are low-cost, highly biocompatible, and mechanically robust; however, they lack permeability and flexibility. The current trend is that glass/silicon and PDMS are commonly used for laboratory research, common thermoplastics are suitable for standardization and commercial mass production of organ chips, and hydrogels are more suitable for biological applications. This difference leads to different preferences, which call for a combination of different materials to build more powerful microfluidic equipment to achieve specific goals. In addition, cells that form organoids lack normative sources and are prone to genetic variation, leading to the variable ori-

Table 1. (Continued)

Microfluidic systems	Major properties	Cell sources	Organoid types	Applications
Microfluidic devices with electrochemical or imaging units	• Monitoring the cultured organoids in real-time • Great potential in automated manipulation • Improving nutrient/waste exchange • Recording the electrophysiological signals of organoids in situ	hiPSCs	• Brian[103]	N.A.
		hESCs	• Brain[101,104,105] • Heart[99]	• Modeling the development of brain with appearance of surface wrinkles[104] • Modeling spinal nociceptive circuitry[101] • Studying the spatial identities within brain organoids[105] • Monitoring damage to cardiac organoids[99]
		Primary human hepatocytes	• Liver[98]	• Evaluating drug toxicity and pharmacokinetics[98]
		Human cancer stem cells	• Breast[69]	• Monitoring the metabolites of oxygen, lactate, and glucose[69]
		Human tumor biopsies	• Intestine[100]	• Analyzing oxygen metabolism in vascularized organoids[100]
		Mouse tongue tissues	• Taste Bud[102]	• Studying taste sensations and taste bud functions[102]
Microfluidic device with interlinked compartments	• Prolonging the organoid culture time • Providing controllable microenvironment • Enabling dynamic interplay among different organs	hiPSCs	• Brain[91,92] • Liver[71] • Heart[91,95,97] • Pancreas[71]	• Probing prodrug metabolism and reciprocal toxicity[91] • Modeling the development and electrophysiological functions of brain[92] • Modeling type 2 diabetes[71] • Drug compound screening[95,97]
		hESCs	• Brain[93]	• Modeling the brain aging driven by immune[93]
		Human ASCs	• Brain[91] • Testis[91,97]	• Probing prodrug metabolism and reciprocal toxicity[91] • Drug compound screening[97]
		Human primary cells	• Brain[91,97] • Liver[91,95–97] • Kidney[96] • Lung[91,95,97] • Testis[91,97]	• Probing prodrug metabolism and reciprocal toxicity[91] • Drug compound screening[95,97] • Studying the therapeutic effects of mesenchymal stromal cell-derived extracellular vesicles[96]
		Human tumor biopsies	• Intestine[94]	• Anti-tumor drug testing[94]

gins of organoids. By taking advantage of gene editing methods (e.g., CRISPR-Cas9) and microfluidic techniques for intracellular delivery,[130] more stable and uniform genotype of cells can be produced. In addition, the high-throughput screening capability of microfluidics can accelerate the construction of standardized cell banks.

In addition, organoid systems as in vitro models have preliminarily demonstrated their application prospects, mainly in fundamental research and drug development. To fulfill the great potential of organoids in biomedical applications, more efforts must be devoted to the organoid field. For example, artificial intelligence (AI) and machine learning can be developed for drug discovery and multiple disease diagnoses, which are appropriate for data analysis of massive organoids generated in dynamic bioreactors. Looking into the future of the field of organoids, there is still significant room for improvement, with the potential of microfluidic technologies having a significant impact. We envision that the convergence of various approaches from biomaterials, engineering, and stem cell biology will greatly improve our understanding of the dynamic processes of organ development and human physiopathology, thereby advancing translational medicine research. It also calls for the joint efforts of scientists from multidisciplinary fields involving materials science, microscale technology, tissue engineering, and development biology.

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Conflict of Interest

The authors declare no conflict of interest.

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