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Complex *in vitro* 3D models of digestive system tumors to advance precision medicine and drug testing: Progress, challenges, and trends

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ABSTRACT

Digestive system cancers account for nearly half of all cancers around the world and have a high mortality rate. Cell culture and animal models represent cornerstones of digestive cancer research. However, their ability to enable cancer precision medicine is limited. Cell culture models cannot retain the genetic and phenotypic heterogeneity of tumors and lack tumor microenvironment (TME). Patient-derived xenograft mouse models are not suitable for immune-oncology research. While humanized mouse models are time- and cost-consuming. Suitable preclinical models, which can facilitate the understanding of mechanisms of tumor progression and develop new therapeutic strategies, are in high demand. This review article summarizes the recent progress on the establishment of TME by using tumor organoid models and microfluidic systems. The main challenges regarding the translation of organoid models from bench to bedside are discussed. The integration of organoids and a microfluidic platform is the emerging trend in drug screening and precision medicine. A future prospective on this field is also provided.

Contents

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; ALI, Air liquid interface; BE, Barrett esophagus; CAFs, cancer-associated fibroblasts; CC, cholangiocarcinoma; CRC, colorectal cancer; DC, dendritic cells; GC, gastric cancer; GI, gastrointestinal; HAN, high-affinity neoantigens; HCC, hepatocellular carcinoma; hHSC, human hematopoietic stem cells; hiPSCs, human-induced pluripotent stem cells; hPBMC, human peripheral blood mononuclear cells; IFN-DCs, interferon-α-conditioned dendritic cells; mCRC, metastatic CRC; MDSC, myeloid-derived suppressor cells; NK, natural killer; OOAC, organ-on-a-chip; OTSC, organotypic tumor slice culture; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; PDO, patient-derived organoids; PDX, Patient-derived xenograft; TCR, T cell receptors; TCR-T, T cells engineered to express tumor-specific TCRs; TILs, tumor-infiltrating lymphocytes; TIME, tumor immune microenvironment; TME, tumor microenvironment.

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1. Introduction

Cancer has become *one of the* major threats to *human health* and *the* morbidity of cancers keeps increasing rapidly, accounting for nearly 10 million deaths in 2020 (Sung et al., 2021). Digestive system cancers account for nearly half of all cancers around the world and have a high mortality rate. Cancer research includes the dissection of tumorigenesis, the identification of new drug targets, and the development of novel therapeutic agents. It relies heavily on the successful application of cancer models such as two-dimensional (2D) cell cultures and animal models. With the development of precision medicine, 2D cell culture models can hardly meet the requirements of biomedical research. The application of 2D cell lines enabled researchers to study the pathogenesis and progression of tumors easily and cost-efficiently. But cell lines lack tumor microenvironment (TME), including immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, nerve fibers, extracellular matrix components, and three-dimensional (3D) architecture. Moreover, the phenotype and genetic information of the cells after passages are significantly different from those of their parental cells (Olivotto & Dello Sbarba, 2008; Sambuy et al., 2005). In addition, 2D cell lines obtained from a small part of the whole tumor tissue, cannot fully characterize the tumors since the heterogeneity of cell components and genetic information are lost (Stein, Litman, Fojo, & Bates, 2004; Szakács & Gottesman, 2004). Another limitation of the 2D cell culture model is associated with the fact that cross-contamination of cell lines might compromise research results and affect scientific reproducibility (Yu et al., 2015).

In recent years, the cell line-derived xenograft (CDX) mouse model is the most widely used animal model in cancer research. However, the predictive power of CDX in evaluating the clinical therapeutic response is poor due to the lack of cells directly derived from the tumor biopsies, and the derivation of immortalized cell line itself is hard to standardize. These limitations severely limit the applications of these models in conducting more extensive and in-depth mechanistic research on tumorigenesis and achieving personalized drug screening (Lallo, Schenk, Frese, Blackhall, & Dive, 2017).

Patient derived xenograft (PDX) mouse model, which simulates the *in vivo* microenvironment, is generally believed to be a good model for cancer research, drug development, and preclinical evaluation (Gao et al., 2015; Tentler et al., 2012). Nevertheless, there are several shortcomings in using PDX models: (1) There is a mismatching between the establishment of PDX model and the clinical schedule of patient treatment. PDX models require significant time for tumor expansion, and often with reduced tumor take rates and high costs. These disadvantages limit PDX for real-time personalized drug application (Kim et al., 2009; Tentler et al., 2012). (2) PDX models are often lack of immune-microenvironment and may be not suitable for immune therapy application. To avoid the response from the animal's immune system, the patient-derived tumor is often transplanted into severe immune-deficient mice (Hidalgo et al., 2014). For this reason, PDX models have limited utility in screening for immune mediating agents such as immune checkpoint inhibitors (*e.g.* anti-PD1), or agents that act by activating immune elements such as anti-CD40 antibodies. (3) There may be inconsistence between the experimental results on PDX models and the clinical outcomes, due to the genetic drift from the primary (F0) tumor (Gao et al., 2015). And (4) The use of animals in PDX models still faces many ethical riddles.

Therefore, better simulating the interaction between cancer cells and TME, improving the therapeutic efficiency in precision oncology, and promoting the translation from bench to bedside have been the main issues needed to be addressed by researchers, worldwide. Scientists have been trying to explore and develop cost-effective, fast, simple, accurate, and high-throughput preclinical models, and several achievements have been made in the last few years, fortunately.

The 3D cell culture models, in agreement with the 3R strategy to "reduce, refine, and replace" animals in experimental testing, have gradually become a hot spot in cancer research and a reliable alternative to 2D cell culture and animal models in personalized medicine and drug screening (Fig. 1). Malignant tumors of the digestive system, including esophageal cancer, gastric cancer (GC), colorectal cancer (CRC), liver cancer, and pancreatic cancer, are the most common malignant tumors (Sung et al., 2021), coming only after breast and lung cancer. The latest research progresses of 3D cell culture models combined with TME in malignant digestive system tumors is reviewed herein. Regarding the inclusion criteria of organoids, the authors searched for the studies on digestive system tumor organoids in PubMed and included representative studies that had successfully established tumor organoid lines from >4 patients. About tumor-on-chip models, we searched for studies focusing on construction of tumor microenvironment. Studies without the full text, lack of credible argument and reproducibility were excluded.

2. Organoid models: Design, culture, and applications

The organoid model is a type of 3D *in vitro* model that has emerged in recent years. Lancaster and Knoblich defined an organoid as a 3D multi-cell assembly which contained several cell types that develop from stem cells or organ progenitors and self-organize through cell sorting and spatially restricted lineage commitment, similar to the process *in vivo* (Lancaster & Knoblich, 2014). The structure of organoids is similar to that of its parental organs and has the following key characteristics: 1) Present multiple organ-specific cell types; 2) Possess some of the key organ-specific functions, such as excretion, filtration, neural activity, and contraction; 3) The cells within the organoid group are spatially organized similar to its parental organ (Clevers, 2016; Lancaster & Knoblich, 2014).

2.1. Design of organoid models of digestive system cancers

The earliest digestive system cancer organoid model was established by Sato, et al. in 2011 (Sato et al., 2011). They improved the mouse small intestine organoid culture condition and successfully established the first human CRC organoid model (Sato et al., 2011). Different from the organoids derived from the mouse intestinal adenoma, human CRC organoids usually grew as irregular compact structures rather than as simple cystic structures. Since then, GC organoids, pancreatic cancer organoids, and liver cancer organoids have been successfully established (Table 1). In addition, *our team also established a* patient-derived organoids (PDOs) biobank of digestive cancers which can be *applied to* extensive studies (Li et al., 2021).

2.2. Culture systems

The key advantage of organoid culture is to provide a culture system that maximally simulates the *in vivo* physiological microenvironment to

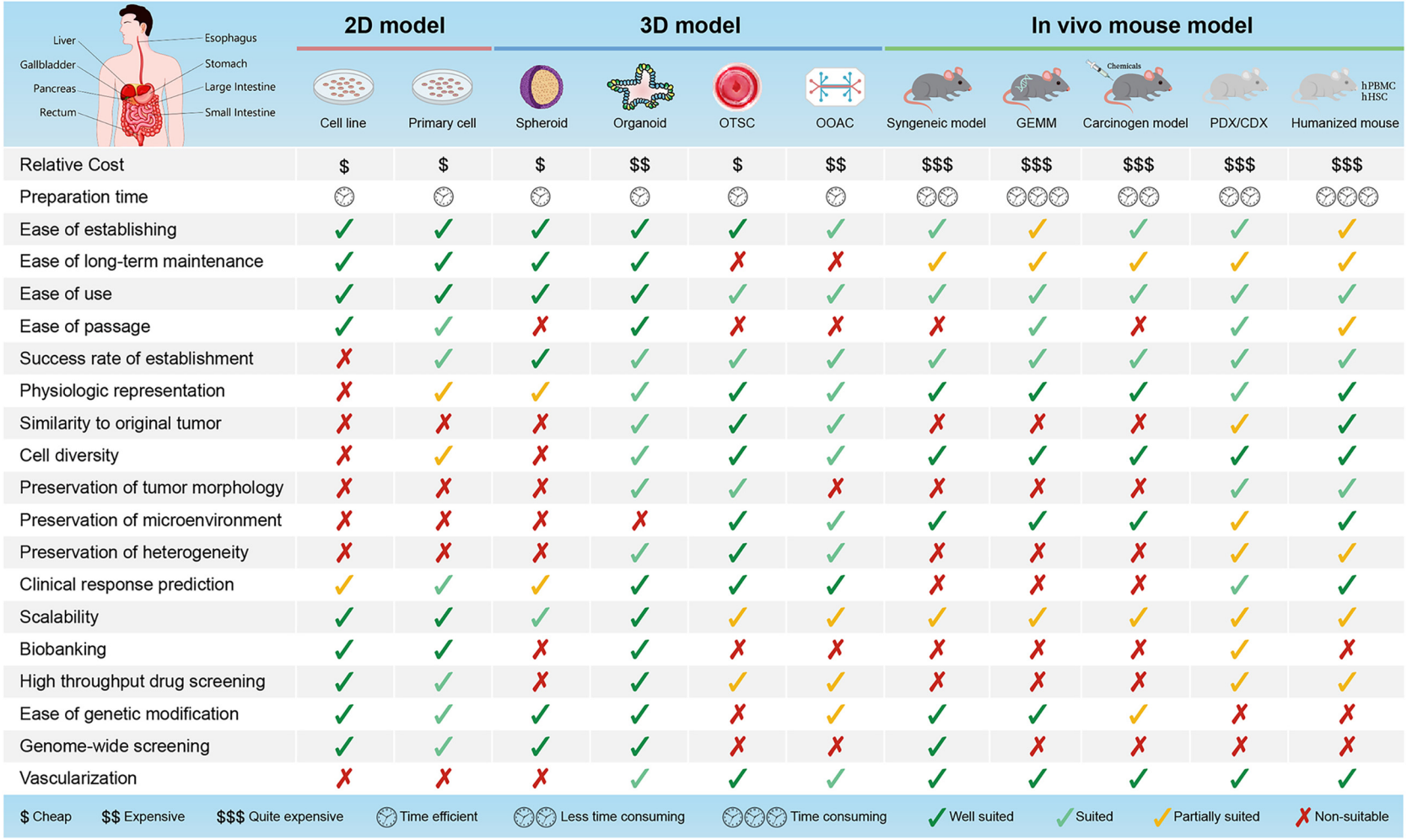


Fig. 1. Comparisons of current models for translational cancer research. For each model, $ represents the relative cost to establish and maintain the model. ◷ represents the relative preparation time to establish and maintain the model. The dark green ✓ indicates well suited; the light green ✓ indicates suited; the yellow ✓ and yellow ✓ indicates partially suited; the red × indicates non-suitable. OTSC: organotypic tumor slice culture; OOAC: organ-on-a-chip; hPBMC: human peripheral blood mononuclear cells; hHSC: human hematopoietic stem cells.

Table 1
Representative of published human PDO biobanks of digestive cancers[a].

Study information[b]	Modifications to commonly used PDO culture medium[c]	Key findings[d]
Esophageal cancer		
Liu X, et al. 2018 (Liu et al., 2018). USA. Endoscopy. 7 BE.	(+): SB202190, PGE2. (−): GlutaMAX.	1. PDOs simulated neoplasia *via* genome editing. 2. Wnt/β-catenin signaling pathway contributed to the neoplastic transformation.
Derouet MF, et al. 2020 (Derouet et al., 2020). Canada. Endoscopy. 16 EAC.	(+): CHIR, SB202190. (−): FGF10, NAM, Y-27632.	1. PDOs recapitulated features of parental tumors. 2. PDOs showed similar drug response to patients in the clinic.
Karakasheva TA, et al. 2020 (Karakasheva et al., 2020). USA. Surgery or endoscopy. 15 ESCC.	(+): N2. (−): Wnt3A, FGF10, NAM, Gastrin, A83–01.	PDOs recapitulated parental tumor features.
Gastric cancer		
Yan HNN, et al. 2018 (Yan et al., 2018). China. Surgery. 46 GC.	(+): None. (−): NAM.	1. A gastric cancer organoid (GCO) biobank which contained the most known molecular subtypes was established. 2. PDOs biobank can be used for large-scale drug screening.
Nanki K, et al. 2018 (Nanki et al., 2018). Japan. Surgery, endoscopy, ascites. 36 GC.	(+): Nutlin-3. (−): NAM, Y-27632.	1. A biological library of genetically engineered gastric organoids and PDO lines was established. 2. Organoids can be used for investigating the function of driver gene mutations.
Seidlitz T, et al. 2019 (Seidlitz et al., 2019). Germany. Surgery. 20 GC.	(+): N2. (−): Y-27632.	1. Human and mouse GCOs were established. 2. GCOs recapitulated typical characteristics and altered pathways of human GC.
Steele NG, et al. 2019 (Steele et al., 2019). USA. Surgery. 7 GC.	(+): N2. (−): GlutaMAX, A83–01.	PDOs recapitulated features and drug sensitivity patterns of parental tumors.
Ukai S, et al. 2020 (Ukai et al., 2020). Japan. Surgery. 10 GC.	(+): None. (−): NAM.	1. Four 5-FU-resistant GCOs were established. 2. KHDRBS3 mediated 5-FU resistance in 5-FU resistant PDOs.
Akira Ishikawa A, et al. 2020 (Ishikawa et al., 2020). Japan. Surgery. 10 GC.	(+): None. (−): NAM.	1. PDOs demonstrated a mucin phenotype. 2. For studying the biology of GC, Organoids are effective tool for studying the biology of GC.
Togasaki K, et al. 2021 (Togasaki et al., 2021). Japan. Surgery, ascites. 7 GC.	(+): None. (−): NAM.	1. Organoids structures could be transformed by removing Wnt and R-spondin from the culture medium. 2. Phenotypes of diffuse GC may be influenced by TME.
Colorectal cancer		
Wetering MVD, et al. 2015 (van de Wetering et al., 2015). Netherlands. Surgery. 20 CRC.	(+): SB202190, PGE2. (−): FGF10, A83–01.	1. A CRC PDOs biobank was established and the PDOs represented major CRC molecular subtypes. 2. PDOs allow the detection of gene-drug associations.
Weeber F, et al. 2015 (Weeber et al., 2015). Netherlands. Needle. 14 mCRC.	(+): SB202190, PGE2. (−): Wnt3A, FGF10, Y-27632.	1. Organoid cultures can be established from biopsies of mCRC. 2. PDOs of mCRC recapitulated genetic features of metastasis.
Fujii M, et al. 2016 (Fujii et al., 2016). Japan. Surgery, endoscopy. 43 CRC.	(+): SB202190. (−): FGF10, NAM, Y-27632.	1. PDOs recapitulated histopathological grade and differentiation capacity of their parental tumors. 2. Niche-independent growth is associated with the adenoma-carcinoma transition reflecting the accumulation of multiple mutations.
Cristobal A, et al. 2017 (Cristobal et al., 2017). Netherlands. Surgery. 7 colon cancer.	(+): SB202190, PGE2. (−): GlutaMAX, FGF10, Y-27632.	Each patient showed a personalized organoids proteome profile.
Ooft SN, et al. 2019 (Ooft et al., 2019). Netherlands. Endoscopy. 34 mCRC.	(+): SB202190, PGE2. (−): GlutaMAX, Wnt3A, FGF10, Gastrin, Y-27632	1. PDOs of mCRC could be used to predict the patients' response to irinotecan-based therapies. 2. PDOs failed to predict the outcome for therapies with 5-fluorouracil plus oxaliplatin.
Ganesh K, et al. 2019 (Ganesh et al., 2019). USA. Endoscopy. 41 Rectal cancer.	(+): SB 202190, N2. (−): FGF10, Y-27632.	1. PDOs recapitulated features and chemoradiation sensitivity patterns of parental tumors. 2. PDO combined with animal models can be used for cancer biology and drug sensitivity research.
Yao Y, et al. 2020 (Yao et al., 2020). China. Endoscopy. 96 Rectal cancer.	(+): SB202190, PGE2, N2. (−): Wnt3A, FGF10, Y-27632.	1. An organoid biobank from patients with locally advanced rectal cancer has been generated. 2. PDOs recapitulated features and neoadjuvant chemoradiation sensitivity patterns of parental tumors.
Narasimhan V, et al. 2020 (Narasimhan et al., 2020). Australia. Surgery, endoscopy. 19 CRC.	(+): SB202190, SB431542. (−): Wnt3A, RSPO1, Noggin, FGF10, NAM	1. Organoids were established from peritoneal metastases. 2. Drug sensitivity testing of PDOs can help guide novel treatment choices in the poor diagnosis cohort.
Yan HHN, et al. 2020 (Yan et al., 2020). China. Surgery, endoscopy. 20 CRC.	HISC. See Ref (Yan et al., 2020).	Rare genetic profiles were revealed in sporadic early-onset CRC PDOs.
Hepatocellular carcinoma		
Broutier L, et al. 2017 (Broutier et al., 2017). Netherlands, UK. Surgery. 3 CC, 3 HCC, 2 CHC.	(+): N2, HGF, FSK.	1. PDOs recapitulated features of parental tumors. 2. ERK inhibitor SCH772984 was identified as a potential therapeutic drug.
Nuciforo S, et al. 2018 (Nuciforo et al., 2018). Switzerland. Needle. 8 HCC, 3 CC.	(+): N2, HGF, FSK. (−): GlutaMAX, Noggin, Y-27632.	1. PDOs recapitulated features and genetic heterogeneity of parental tumors. 2. PDOs could be used to test the sensitivity of sorafenib.
Li L, et al. 2019 (Li et al., 2019). USA. Surgery. 2 HCC, 3 CC.	(+): N2, HGF, FSK. (−): GlutaMAX.	1. Intratumor drug response heterogeneity was demonstrated *via* PDOs. 2. A subset of drugs, which are FDA approved for indications other than liver cancers, appeared pan-effective in the majority of these PDOs.
Fong ELS, et al. 2018 (Fong et al., 2018). Singapore. 14 PDX lines.	3D sponge culturing. See Ref (Fong et al., 2018).	1. Organoids derived from HCC-PDX recapitulated features of parental tumors. 2. Organoids derived from HCC-PDX could use for drug testing.

Table 1 (*continued*)

Study information[b]	Modifications to commonly used PDO culture medium[c]	Key findings[d]
Wang S, et al. 2020 (Wang et al., 2020). China. Surgery. 4 HCC.	(+): N2, HGF, FSK, dexamethasone. (−): Wnt3A, RSPO1, Noggin.	1. HCC PDOs well maintained the histological features and drug sensitivity patterns of parental tumors. 2. Sorafenib resistance in $CD44^+$ PDOs could be reversed by a Hedgehog signaling inhibitor.
Zhao X, et al. 2020 (Zhao et al., 2020). China. Surgery. 4 HCC.	(+): N2, HGF, FSK.	$ACADL^{low}$ PDOs growth was suppressed by verteporfin.
Leung CON, et al. 2020 (Leung et al., 2020). China. Surgery. ≥4 HCC.	(+): N2, HGF, FSK. (−): GlutaMAX	Sorafenib resistance can be reversed by SHP099 in HCC PDOs.
Li L, et al. 2021 (Li et al., 2021). USA. Surgery, needle. 20 HCC.	(+): N2, HGF, FSK.	1. HCC PDOs were sensitive to omacetaxine. 2. Omacetaxine suppressed HCC PDOs growth by targeting PLK1.
Pancreatic cancer		
Boj SF, et al. 2015 (Boj et al., 2015). USA, Netherlands. Surgery, endoscopy. 10 PDAC.	(+): PGE2. (−): Y-27632	1. PDOs recapitulated the ductal- and disease-stage-specific features of parental tumors. 2. In murine pancreatic organoids, transcribing and proteomic analyses revealed genes and pathways altered during disease progression.
Tiriac H, et al. 2018 (Tiriac et al., 2018). USA. Surgery, needle. 60 PC.	(+): PGE2. (−): Y-27632	1. PDOs recapitulated features and drug sensitivity patterns of parental tumors. 2. PDOs enabled longitudinal evaluation of synchronous metastases.
Seino T, et al. 2018 (Seino et al., 2018). Japan. Surgery, needle, ascites. 49 PC.	(+): SB202190. (−): FGF10, NAM, Y-27632.	Functional heterogeneity of Wnt niche is non-genetically formed through tumor progression.
Tiriac H, et al. 2018 (Tiriac et al., 2018). USA. Needle. 33 cases.	(+): PGE2. (−): Y-27632.	PDOs were established from endoscopic ultrasound-guided fine needle biopsies (FNB).
Driehuis E, et al. 2019 (Driehuis et al., 2019). Netherlands. Surgery, endoscopy. 52 cases.	(+): PGE2. (−): Y-27632.	1. PDOs recapitulated tumor histology and contain genetic alterations typical of parental tumors. 2. PDOs could be used for screening novel therapeutics and confirmed that PRMT5 inhibition effectively targets $MTAP^-$ tumors.
Bian B, et al. 2019 (Bian et al., 2019). France. Surgery, needle. 24 PDAC.	No modification.	1. PDAC-derived organoids can be established from PDTX and EUS-Guided Fine-Needle Aspiration (EUS-FNA) biopsies. 2. NHWD-870 or JQ1 was more efficient in MYC^{high} PDOs.
Hennig A, et al. 2019 (Hennig et al., 2019). Germany. Surgery, needle. 31 PDAC.	(+): N2. (−): Y-27632.	1. PDAC-organoids can be categorized into known subtypes based on KRT81 and CFTR immunoreactivity. 2. PDOs recapitulated subtype-specific immunoreactivity of parental tumors.
Seppälä TT, et al. 2020 (Seppälä et al., 2020). USA. Surgery, endoscopy. 59 PDAC.	(+): PGE2. (−): Y-27632.	1. PDOs could be established from specimens obtained remotely and maintain the clonal heterogeneity. 2. PDOs can guide postoperative adjuvant chemotherapeutic selection.
Oni TE, et al. 2020 (Oni et al., 2020). USA. Sources unclear. 5 PDAC.	(+): PGE2. (−): Y-27632.	SOAT1 was identified as a potential therapeutic target in PDAC.
Raimondi G, et al. 2020 (Raimondi et al., 2020). Spain, Netherlands. Surgery. 7 PDAC.	(+): None. (−): Y-27632	1. Organoids derived from PDAC were specifically infected and lysed by oncolytic adenoviruses (OA). 2. Pancreatic PDOs can be used to predict OA sensitivity.
Huang W, et al. 2020 (Huang et al., 2020). USA. Surgery. 23 PDAC.	Not clear. See Ref (Huang et al., 2020).	1. PDAC organoids exhibited two morphologically defined invasive phenotypes, mesenchymal and collective. 2. SMAD4 mutation determined the invasion phenotypes of PDOs.
Juiz N, et al. 2020 (Juiz et al., 2020). France. Needle. 20 cases.	No modification.	High heterogeneity of PDAC revealed by single-cell sequencing of PDOs.
Vaes RDW, et al. 2020 (Vaes et al., 2020). Netherlands. Surgery. 8 PC.	No modification.	1. PDOs recapitulated the pathophysiological and cachexia-related features of original tumors. 2. The tumor organoids of cachectic patients secreted more GDF-15 and IL-8 than those of non-cachectic patients.
Miyabayashi K, et al. 2020 (Miyabayashi et al., 2020). USA. Surgery, needle. 16 PDAC.	(+): None. (−): Y-27632.	1. PDOs were grafted into mice intraductal to model the progressive switching of PDAC subtypes. 2. A suite of RAS-regulated secreted and membrane-bound proteins that may represent potential candidates for therapeutic intervention in PDAC patients was identified.
Lacomb JF, et al. 2021 (Lacomb et al., 2021). USA. Needle. 56 PDAC.	Not mentioned. See Ref (Lacomb et al., 2021).	PDOs from single-pass FNB biopsies were safe and feasible.
Beato F, et al. 2021 (Beato et al., 2021). USA. Surgery. 13 IPMNs.	(+): PGE2.	Organoids derived from both fresh and cryopreserved IPMN tissues recapitulated features of parental tumors.
Below CR, et al. 2021 (Below et al., 2021). UK. Surgery, needle. 4 PDAC.	(+): PGE2. (−): Y-27632.	1. A fully synthetic hydrogel extracellular matrix was used in organoid cultures. 2. Laminin–integrin α3/α6 signaling was important for PDOs growth.
Gastroenteropancreatic neuroendocrine neoplasm (GEP-NEN)		
Kawasaki K, et al. 2020 (Kawasaki et al., 2020). Japan. Surgery, endoscopy, needle. 3 NET, 16 NEC.	(+): FGF-2, IGF-1. (−): FGF10, NAM, Y-27632.	A PDOs biobank of GEP-NEN was established and recapitulated parental tumor features.
Mixed cancers		
Sato T, et al. 2011 (Sato et al., 2011). Netherlands. Surgery, endoscopy. 20 colon cancer, 5 colonoscopies, 5 BE.	See Ref (Sato et al., 2011).	PDOs could be derived from colon cancer and Barrett's esophagus.
Kijima T, et al. 2019 (Kijima et al., 2019). Japan, USA. Endoscopy. 11 ESCC. 4 OPSCC.	(+): N2, SB202190. (−): GlutaMAX, FGF10.	1. PDOs recapitulated the histopathology features and chemoradiation therapy patterns of original tumors.

(*continued on next page*)

Table 1 (*continued*)

Study information[b]	Modifications to commonly used PDO culture medium[c]	Key findings[d]
		2. CD44^{high} and autophagy were associated with PDOs formation capability and 5-fluorouracil resistance.
Saito Y, et al. 2019 (Saito et al., 2019). Japan. Surgery. 6 BTC, including 3 IHCC, 1 PDAC, 1 GBC, 1 NEC.	(+): N2, FSK. (−): Wnt3A, Noggin, FGF10	1. PDOs recapitulated the histopathology and gene expression features of original tumors. 2. Prognostic biomarkers and potential therapeutic drugs were identified based on PDOs.
Vilgelm AE, et al. 2020 (Vilgelm et al., 2020). USA. Needle. 8 CRC, 5 PDAC, 1 CC, 2 GC, 1 appendiceal carcinoma.	Semi-solid and Disc fine needle aspiration-PDO (FNA-PDO) culture. See Ref (Vilgelm et al., 2020).	Organoids could be derived from several types of tumor tissues obtained by FNA.
Sharick JT, et al. 2020 (Sharick et al., 2020). USA. Surgery. 14 PC, 13 BCE.	DMEM/F-12, FBS, Y-27632, EGF, RSPO1. See Ref (Sharick et al., 2020).	1. Optical metabolic imaging (OMI) was used to capture the cellular heterogeneity of PDOs. 2. OMI of PDOs agreed with long-term therapeutic response in patients.
Shiihara M, et al. 2021 (Shiihara et al., 2021). Japan. Surgery. 6 GBC, 2 PVC, 11 BDC, 1 IPNB, 2 IPMN, 8 PDAC.	(+): N2, PGE2, dexamethasone.	1. PDOs recapitulated the histopathological features and genomic aberrations of parental tumors. 2. Growth of PDOs could be suppressed by ILK inhibitor.

Notes: a, representative biobanks and only organoid biobanks derived from tumor tissues or ascites are included here. b, the study information includes the following details: references, countries, tissue sources (*e.g.*, surgery, endoscopy, needle biopsy/aspiration, and ascites puncture), and patient cases (only for successful organoid derivation). c, the commonly used PDO culture medium is defined as Advanced DMEM/F12, HEPES, GlutaMAX, Antibiotics, B27, HGF (Hepatocyte growth factor), PGE2 (Prostaglandin E2), FSK (Forskolin), NAC (N-Acetylcysteine), Wnt3A, RSPO1 (R-spondin1), Noggin, EGF (Epidermal growth factor), FGF10 (Fibroblast growth factor-10), NAM (Nicotinamide), Gastrin, A83–01 (TGFβi), Y-27632 (RHOKi). d, tumor features mean the morphological, histological, phenotypical, pathophysiological, pharmacological, molecular, transcriptomic, and genomic or functional features of parental tumors, details can be obtained from corresponding references.
Abbreviation: BC, breast cancer. BDC, bile duct carcinoma. BE, Barrett esophagus. BTC, biliary tract carcinoma. CC, cholangiocarcinoma. CHC, combined HCC/CC. CRC, colorectal cancer. EAC, esophageal adenocarcinoma. ESCC, esophageal squamous cell carcinoma. GBC, gallbladder cancer. GC, gastric cancer. HCC, hepatocellular carcinoma. IHCC, intrahepatic

induce stem cells to differentiate into specific tissue and show specific organ morphology *in vitro* (Saglam-Metiner, Gulce-Iz, & Biray-Avci, 2019). Organoid culture systems contain growth factors, such as EGF, R-Spondin, and Noggin, that are necessary for the proliferation and differentiation of stem cells (Sato et al., 2009). More importantly, the culture system of organoids must contain extracellular matrix (ECM) for maintaining both the 3D biochemical and biophysical cues of the native environment of organoids and the polarity of the cell arrangement (Xu et al., 2001). Matrigel® is one of the most popular protein-based hydrogels, which mainly contains laminin, collagen IV, and heparan sulfate proteoglycan (Bissell, Arenson, Maher, & Roll, 1987; Kleinman et al., 1982). Generally, for the culture of organoids, cells or biopsies are mechanically/enzymatically digested and then single cells are suspended in Matrigel®. When the Matrigel® is solidified, the appropriate medium is added, and the cells are cultivated in the common incubator and passaged every 1–2 weeks. Successfully established organoids can be cryopreserved and thawed like cell lines (Drost et al., 2016).

2.3. Applications of tumor organoids in the digestive system

2.3.1. Basic science and mechanistic research

The primary GC organoid biobank established by Yan et al. (Yan et al., 2018) contained the most known molecular subtypes, including microsatellite instability (MSI), Epstein-Barr virus (EBV), intestinal (chromosome instability, CIN), and diffuse (genomically stable, GS) subtypes, with *CLDN18-ARHGAP6* or *CTNND1-ARHGAP26* fusions or *RHOA* mutations. It also captured regional heterogeneity and subclone architecture. Interestingly, *CLDN18-ARHGAP6* fusion transcript was detected in one of the established organoids, and this is also the first report of *in vitro* model carrying these specific molecular pathway change (Yan et al., 2018). Compared to previous research works, they described GC organoids with *CLDN18-ARHGAP6* and *CTNND1-ARHGAP26* fusions, EBV expressions, and *RHOA* mutations. So far, cell and organ models with these mutations have rarely been reported. GC organoid biobank containing comprehensive genetic data will help us learn more about the relationship between the genotypes and the phenotypes of the organ, and find more driving genes and therapeutic targets for GC.

Organoids are amenable to genome editing. Drost and colleagues edited the genome in organoids *in vitro*, simulated the process of CRC carcinogenesis and progression, and re-proved the functions of key driving genes in CRC occurrence and development in organoids (Drost et al., 2015). In their study, the four most common mutations (*APC, TP53, KRAS, and Smad4*) were introduced into organoids derived from human intestinal stem cells cultured *in vitro via* CRISPR/cas9 technology. It was found that the quadruple mutant organoids grew into tumor with invasive characteristics in the transplanted mice, while the triple mutant organoids in which three of the four genes were mutated grew with relatively limited invasive characteristics. The tumors contained large cysts which showed the characteristics of well-differentiated cancer. For double mutant (*APC* and *p53*) organoids, extensive aneuploidies were found in mice, indicating that these two mutations were sufficient to induce carcinogenesis (Drost et al., 2015). Matano, et al. (Matano et al., 2015) also generated normal intestinal epithelial organoids with mutations in tumor-related pathways and genes *via* CRISPR/Cas9 technology. The mutated organoids were transplanted into the kidney subcapsular space in immunodeficient mice. It was observed that organoid transplants eventually formed tumors in mice. In addition to CRISPR/Cas9, shRNA technology can also be used for gene editing in tumor organoids (Nadauld et al., 2014). Since organoids show the 3D structure and usually contain multiple cell types from their original organ, genomic modified organoids can be used to better recapitulate the development and phenotypic changes from normal tissues to tumors at the "organ level". Genome editing in organoids provides new insights into the histopathological transformation process during tumorigenesis, thus allowing us to understand the fundamental role of driver genes and pathways in tumor pathogenesis. This knowledge may open up new avenues for identifying novel early tumor biomarkers and tumor therapeutic targets.

2.3.2. Drug screening and personalized treatment of digestive system tumors

The high heterogeneity of tumors makes cancer therapy extremely complicated, which was also the reason why tumor treatments need to be personalized (Burrell, McGranahan, Bartek, & Swanton, 2013). Chemo-resistance is a common but unpredictable phenomenon in many patients with digestive system cancers. Therefore, a more reliable and workable preclinical model that precisely predicts patients' responses to personalized treatment is urgently needed. Currently, it is believed that the stronger the ability of the preclinical models to capture sub-clonal populations of parental tumors, the more accurate it will be

in predicting patient drug responsiveness. Due to its ability to preserve the heterogeneity of original tumors, PDX models have become the gold standard for compound testing in oncology (Hidalgo et al., 2014). Compared with other preclinical prediction models, the clonal selection and heterogeneity observed in PDOs are similar to PDX *in vivo* models of cancers, which may be used for drug screening more efficient, convenient, and economical *in vitro* (Eirew et al., 2015; van de Wetering et al., 2015; Weeber et al., 2015).

A biobank comprised of PDOs and PDXs that derived from 106 patients and represented all CRC subtypes was established (Schutte et al., 2017). In most cases, PDOs and PDXs could recapitulate the genetic landscapes of parental tumors. In the biobank, 19 tumors were modelled in both PDOs and PDXs. Eight drugs were tested in the 19 pairs of PDO/PDX siblings, and the response patterns between the two models were fairly concordant except for AZD8931 and 5-FU, which proved that the effect of PDO on drug prediction might not be inferior to that of PDX (Schutte et al., 2017).

Previously reported studies have demonstrated that tumor organoids could be used to test the sensitivity of chemotherapeutic drugs *ex vivo*, and the results were highly consistent with the PDX models. Nevertheless, more convincing data is still needed to show that organoid testing results could precisely predict the clinical chemotherapeutic outcome of patients. In a report in 2018, researchers recruited patients with metastatic CRC and gastroesophageal cancer who had received extensive pretreatments from a phase 1/2 clinical trial and established a live biobank of PDOs (Vlachogiannis et al., 2018). In that study, 19 cases of PDOs were selected from the biobank for *in vitro* drug sensitivity testing, and compared with the clinical outcomes of the patients with drug responses in their respective organoids (Vlachogiannis et al., 2018). It was found that the sensitivity, specificity, positive predictive value, and negative predictive value of PDOs in predicting the response of patients to targeted drugs or chemotherapy were found to be 100%, 93%, 88%, and 100%, respectively. Another 65 cases of organoids derived from 41 rectal cancer patients have also been established. The organoids retained the histopathological and molecular characteristics of their parental tumor biopsies, and their *ex vivo* responses to clinically relevant chemotherapeutic drugs and radiation treatment correlated with the clinical responses noted in their corresponding patients (Ganesh et al., 2019).

In addition, organoids can also be used to repurpose some non-cancer drugs for cancer treatment. Since these re-purposed drugs have passed toxicity tests and successfully marketed, the time and costs of drug development could be reduced (Zhang et al., 2020). A variety of off-label drugs, such as statins, aspirin, and metformin, have been studied for cancer treatment (Jiang et al., 2020; Lin, Li, Xu, & Mei, 2021; Lv et al., 2020). After establishing a PDAC organoid biobank, Hirt et al. tested 1172 FDA-approved compounds and found 26 compounds to be capable of killing PDAC organoids. They verified the activity of these drugs through *in vivo* experiments and found that emetine and ouabain, two drugs approved for non-cancer indications, can kill PDAC organoids by interfering with the ability of PDAC to respond to hypoxia. But most of these 26 compounds had no killing effect on 2D PDAC cell lines (Hirt et al., 2022).

In brief, the experimental results of a large number of digestive system tumor organoids used in preclinical drug screenings are promising. It is clear that if the drug response in PDOs *ex vivo* could accurately predict the drug responsiveness *in vivo*, we can assist to use in clinics, in the future. Theoretically, patients could achieve accurate and personalized treatment and the prognosis could be improved, thus avoiding the economic burden and side effects caused by the selection of drugs with poor effects. Moreover, compared with the PDX animal model, the PDO system is simple, rapid, and cheap, and can be used as a preclinical model for the screening of various drugs, including anticancer drugs.

2.4. Advantages and limitations of PDO

Compared with 2D cell lines, PDO models retained the genetic heterogeneity of their source tumor tissues and kept the genomic stability for multiple passages. All these characteristics indicated that PDOs performed better in recapitulation of *in vivo* environment (Sasai, 2013). Compared with PDX models, PDOs have the advantages of shorter establishment time, simpler technology, lower cost, higher throughput manipulations, more editable genome, and no animal welfare and ethical issues (Hidalgo et al., 2014).

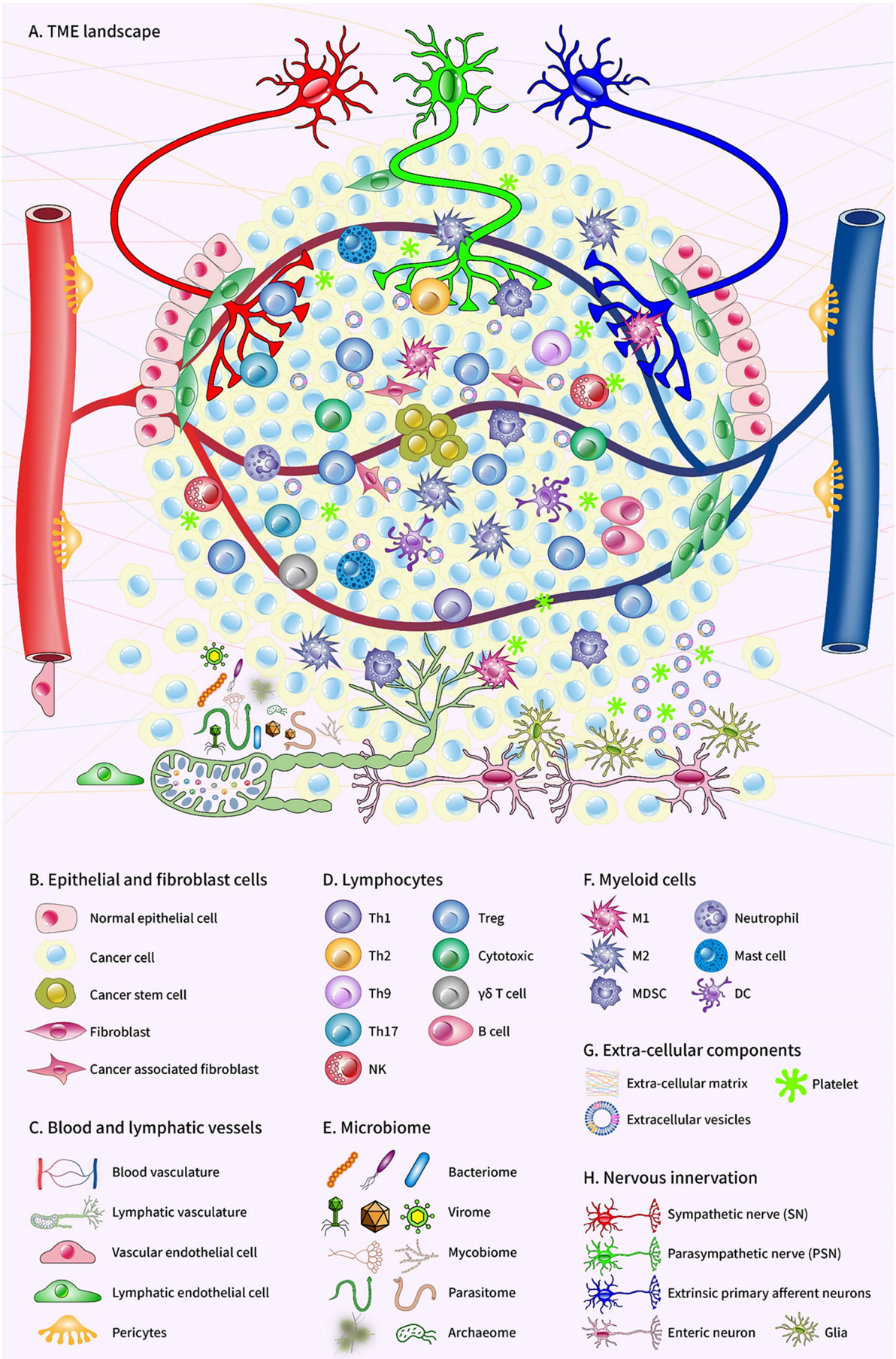
However, it should be noted that PDO models also present limitations. Firstly, there is no standardized tumor organoid culture system currently. The PDO model relies heavily on manual operations, and the standard operation is difficult, and not equal among different labs. Thus, it is not suitable for automation, real-time monitoring and detection, difficult for high-throughput screening, and the test results are hard to repeat. In addition, epithelial cells are still the major cellular components of tumor organoids, while other TME components such as mesenchymal cells, nerve fibers, blood vessels, and immune cells are still lacking in most cases of organoids (Seidlitz et al., 2019; van de Wetering et al., 2015). Therefore, the current reported organoid models cannot fully recapitulate the characteristics and environment of the tumor, making it difficult to study the interactions between tumor and TME and evaluate the efficacy of drugs that target TME. To simulate a more complete tumor with TME, a more complex *ex vivo* model is urgently needed.

3. Engineering TME in tumor organoid model

TME consists of ECM, mesenchymal cells, immune cells, and soluble factors (*e.g.*, cytokines, growth factors, hormones) (Wu & Dai, 2017) as depicted in Fig. 2. With an in-depth understanding of TME, it was found that the composition of the TME, the proportion of the cellular components, and the changes in the activation state of the cells in TME had important impacts on tumor occurrence, progression, metastasis, and drug resistance (Maman & Witz, 2018; Quail & Joyce, 2013). The heterogeneity of TME plays an imperative role in tumor therapy success (Junttila & de Sauvage, 2013; Klemm & Joyce, 2015; Palucka & Coussens, 2016). Therapeutic options that modulate TME have brought good prospects for tumor treatment. However, advanced 3D models that recapitulate the *in vitro* interactions between cancer cells and TME are still required. An increasing number of researchers are seeking to establish TME in tumor organoid models to better simulate the state of the tumor *ex vivo*.

3.1. CAFs in organoid models

Air liquid interface (ALI) culture is a new organoid culture method that strongly supports the growth of organoids in the form of epithelial/mesenchymal hybrids without the addition of exogenous growth factors. Generally, the tissue-containing gel is in the upper transwell insert and exposed to air directly, the culture medium is filled into the outer dish and should not reach above the tissue-containing gel which allows the formation of the ALI microenvironment (Katano et al., 2013; Ootani et al., 2009). Based on this model, Li et al. (Li et al., 2014) successfully cultured mouse pancreas, stomach, and colorectal organoids in 2014. This organoid model includes epithelial/mesenchymal (fibroblasts) cells, and allows for *in vitro* cancer modeling and verification of cancer driver genes in a physiological environment superior to transformed cell lines or primary cultures of epithelial cells were used (Li et al., 2014). To study the classification and role of CAFs in pancreatic cancer, a 3D organoid co-culture system was established by Öhlund et al. (Ohlund et al., 2017). Mouse pancreatic cancer organoids and mouse pancreatic stellate cells were co-cultured in Matrigel® (Ohlund et al., 2017). It was found that the pancreatic stellate cells in the co-culture system were activated from the resting state and differentiated into CAFs which could produce desmoplastic stroma, while the pancreatic stellate cells cultured in Matrigel® alone remained static. CAFs promoted the proliferation of PDAC organoids by means of secreting IL-6. This phenomenon mimicked the mutual interactions between PDAC


A. TME landscape
B. Epithelial and fibroblast cells
Normal epithelial cell
Cancer cell
Cancer stem cell
Fibroblast
Cancer associated fibroblast
C. Blood and lymphatic vessels
Blood vasculature
Lymphatic vasculature
Vascular endothelial cell
Lymphatic endothelial cell
Pericytes
D. Lymphocytes
Th1
Th2
Th9
Th17
NK
Treg
Cytotoxic
γδ T cell
B cell
E. Microbiome
Bacteriome
Virome
Mycobiome
Parasitome
Archaeome
F. Myeloid cells
M1
M2
MDSC
Neutrophil
Mast cell
DC
G. Extra-cellular components
Extra-cellular matrix
Extracellular vesicles
Platelet
H. Nervous innervation
Sympathetic nerve (SN)
Parasympathetic nerve (PSN)
Extrinsic primary afferent neurons
Enteric neuron
Glia

cells and CAFs through paracrine cytokines within the body (Ohlund et al., 2017).

In addition to the ALI model, Liu et al. (Liu et al., 2021) applied transwell to construct a co-culture model of liver cancer organoids and CAFs. They found that CAFs and liver cancer organoids can promote the growth of each other through paracrine signaling. Also, in the presence of CAFs, liver cancer organoids were less sensitive to anticancer drugs including sorafenib, regorafenib, and 5-fluorouracil. In the mouse xenograft model, co-transplantation of organoids with CAFs resulted in much larger tumors as compared with transplanting organoids alone. This study not only co-cultured liver cancer organoids and CAFs, but also transplanted them into immunodeficient mice, which better integrated PDO, TME cells, and animal models, achieving reliable results.

3.2. Immune microenvironment in organoid models

In 2018, Dijkstra et al. (Dijkstra et al., 2018) reported a set of 15 CRC organoids derived from 15 different mismatch repair deficient (*dMMR*) CRC patients. Peripheral blood mononuclear cells (PBMC) that were isolated from patients with *dMMR* CRC were stimulated and activated with autologous tumor organoids every week. Then, the activated and enriched tumor-reactive T cells were co-cultured with PDOs. It was found that the volume of PDOs was reduced and the cancer cells underwent apoptosis extensively. The activity against tumor organoids was restricted to T cells that had been previously subjected to the two-week co-culture with organoids. These T cells did not cause reactivity against normal tissues or mismatch repair proficient (*pMMR*) CRC organoids (Dijkstra et al., 2018). The establishment of this CRC organoid and T cell co-culture platform allows researchers to obtain patient-specific tumor-reactive T cells by co-cultivating peripheral blood lymphocytes (PBL) with matched tumor organoids and producing large numbers of effector T cells targeting the neoplastic cells for adoptive cell transplantations. Secondly, the developed co-culture platform can be used to assess the sensitivity of cancer patients to immunotherapy and explain the potential mechanisms of drug resistance. Such a co-culture platform helps understand the mechanisms of immunotherapy resistance and relapse in cancer patients.

Although Dijkstra et al. have already co-cultured peripheral immune cells with their corresponding CRC organoids, this *in vitro* co-culture platform cannot retain the complexity, diversity, and physical structure of TME. Particularly, it does not allow for the co-culture of primary tumor epithelial cells with their native infiltrating immune populations *en bloc* without reconstitution. Neal et al. (Neal et al., 2018) have tried to mince the tumor tissues obtained from patients diagnosed with colon cancer, pancreatic cancer, bile duct ampullary adenocarcinoma, clear cell renal cell carcinoma (ccRCC), non-small cell lung cancers (NSCLC) and melanoma into small pieces, and then resuspended them in a gel of collagen type I, layered them on the top of pre-solidified gel within an inner trans-well insert to form the air-liquid culture system (Neal et al., 2018). Finally, the transwell insert containing tumor tissues and collagens was placed into a cell culture dish containing regular culture. In that co-culture system, fibroblast and immune cells were also included. The profiling of the immune components found that PDOs contained $CD3^+$ T cells (tumor-infiltrating lymphocytes, TILs), and $CD14^+$ or $CD68^+$ macrophages. Fluorescence-activated cell sorting (FACS) analysis showed that $CD8^+$ (Tc) and $CD4^+$ (Th) T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells and infiltrated $CD3^+$ T cells all expressed PD-1 (Neal et al., 2018). The PDOs faithfully summarized the T cell receptors (TCRs) library of their original tumor biopsy tissues. Then, the PDOs were used to evaluate the response of checkpoint blocker navuzumab within 7 days. TILs within both human and mouse PDOs showed activation, expansion, and cytotoxicity after the addition of navuzumab, and the response rate of PDOs against navuzumab was consistent with that of clinical trials (Neal et al., 2018). That model was able to retain a variety of endogenous immune cell types besides T cells, including macrophages, B cells, and NK cells, and was superior to traditional models where clone amplified or TCR engineered TIL populations are co-cultured with tumor cells.

In addition, Koh et al. (Koh et al., 2021) *co*-cultured GC organoids with immune cells and found that PD-L1-expressing GC organoids did not respond to nivolumab *in vitro* in the presence of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), while GC organoids were sensitive to nivolumab-induced cancer cell death after clearing PMN-MDSCs. Liu et al. (Liu et al., 2021) divided the biopsy specimen from HCC patients into two parts, one for extraction and sorting of TILs, and the other for HCC organoids culturing. The authors co-cultured CD39+/-CD8+ TILs with autologous HCC organoids for 24 h and found that CD39 + CD8+ TILs from high-affinity neoantigens (HAN)-high group induced a superior caspase3/7 positive apoptosis as compared to that observed in the HAN-low group, which is consistent with their finding that HAN value positively correlates with better OS in patients with HCC. These PDO/immune cell co-culture models could better simulate the response of tumor and TME to immunotherapy, promoting the development of immuno-oncology research and personalized immunotherapy.

3.3. Nerve microenvironment in organoid models

Nerve fibers are increasingly recognized as important components of the TME, especially in the GI tract (Drewes et al., 2020; Rao, 2020). A lot of evidence showed that nerve fibers participated in the occurrence and progression of solid tumors in a passive or active manner (Schonkeren, Thijssen, Vaes, Boesmans, & Melotte, 2021; Tan et al., 2021; Zahalka & Frenette, 2020). A few researchers have tried to establish 3D co-culture models of normal GI epithelium and nerve fibers. For example, Workman and others recapitulated the development of a normal enteric nervous system (ENS) by introducing the neural crest cells (NCCs) derived from human-induced pluripotent stem cells (hiPSCs) into the developing human intestinal organoids (HIOs) *in vitro* and found that NCCs migrated to the mesenchyme, differentiated into neurons and glial cells, and displayed neuronal activity (Workman et al., 2017). When the HIOs containing ENS were grown *in vivo* in mice, a glial structure similar to the myenteric and submucosal plexus was formed, functional interstitial cells of Cajal were observed, and electromechanical coupling which regulates the propagation of contraction waves was detected (Workman et al., 2017). Park et al. also used human embryonic stem cells to generate intestinal organoids and successfully co-cultured them with enteric nerves and blood vessels respectively, but failed when they tried to cultivate the intestinal organoids together with enteric nerves and blood vessels together

Fig. 2. The components of TME of digestive system. A) The general landscape of TME of human digestive system. Different cellular components in TME intertwined and interacted with each other to promote the pathogenesis and progression of tumors. B) The digestive system tumors are mainly derived from epithelial cells, cancer cells and cancer stem cells are the basic components of the tumor, CAFs educated by cancers promote the growth of the tumor. C) Microbiota can be found in tumor tissues, especially digestive system tumors, and contribute to the progression of the tumor. The human microbiome usually includes bacteriome, virome, mycobiome, parasitome, and archaeome. D) Cells within the TME secreted macromolecules to form the ECM, secreted small molecules, and released extracellular vesicles (EVs) to communicate with each other. E) Myeloid cells, including macrophages (M1 and M2), myeloid-derived suppressor cells (MDSC), dendritic cells (DC), neutrophils, and mast cells, modulate the anti-tumor immune response of lymphocytes. F) Lymphocytes, especially the cytotoxic $CD8^+$ T cells and the natural killer (NK) cells, are the key immune cells to kill cancer cells, while Treg cells usually suppress the anti-tumor immune response. G) The digestive system tumor receives extrinsic nerve innervations from the sympathetic nerve (SN), parasympathetic nerve (PSN), and extrinsic primary afferent neurons (EPAN). GI tract tumors can further be innervated by intrinsic enteric neurons and glial cells. H) Angiogenesis is frequently found in digestive system tumors to provide nutrients to support the growth of the tumor. Lymphatic vessels are usually found in late-stage tumors and are thought to help the dissemination, invasion, and metastasis of cancer cells.

(Park, Nguyen, & Yong, 2020). In the future, researchers might introduce nerve fibers, an important TME component, into tumor organoids, and have a deeper understanding of the role of nerve fibers in tumor development, as well as screening of new drugs targeting tumor infiltrating nerves.

3.4. Achievements and challenges

As described above, organoids derived from a wide range of clinical sources (surgical specimens, biopsy samples, and malignant ascites) and maintained the heterogeneity of parental tumors after multiple passages. PDO is a good *in vitro* model which can be modelled in a short time, with a high success rate, simple culture operation, and low cost compared with *in vivo* models. It is suitable for large-scale drug screening. Combining PDOs with TME provides us with great prospects in cancer research and drug screening. By co-culturing with TME, PDO models can better simulate the growth status of tumors *in vivo* and make the research conclusions more reliable. Additionally, this strategy can reduce the numbers of animal studies and clinical trials and consequent false positive results, as well as the side effects of ineffective anticancer drugs used in patients. Engineering TME by using PDO models is worth for screening of drugs targeting the TME and guiding the choice of immunotherapy for tumor patients in the clinic.

Although great success has been achieved in *ex vivo* 3D models by introducing TME into digestive system organoids, several limitations still exist. Firstly, due to the lack of vascular structure, blood flow, blood pressure, other biological fluid, and mechanical force stimulations, the current models of tumor organoids introduced by TME components are still not enough to fully simulate the intrinsic TME. Secondly, the tumor organoid model relies heavily on manual operations and presents many reproducibility issues. Finally, all the supplemented external factors that are not necessarily present in the parental tumor may eventually, lead to artificial and misleading findings.

4. Organ-on-a-chip models in microfluidic systems

4.1. Introduction of organ-on-a-chip

Organ-on-a-chip systems are a new *in vitro* 3D cell culture system in which various living cells are seeded on an engineered chip and cultured inside the microfluidic system. Within the organ-on-a-chip system, a variety of cell types, functional tissue interfaces, dynamic physiological fluid flow, mechanical stimulations, and other complex factors derived from the tissue/organ microenvironment can be maintained. Several physiological parameters, including ECM compositions, biochemical gradients, oxygen levels, and biomechanical/electrical cues can be accurately controlled, tackling some of the major organoid culture challenges mentioned above. All these strengths demonstrated that the main structural and functional characteristics of human tissues and organs may be well recapitulated within the organ-on-a-chip system (Bhatia & Ingber, 2014; Reardon, 2015; Vidi et al., 2014; Wlodkowic & Cooper, 2010; Young, 2013). When the organ-on-a-chip model is applied in cancer research, the normal cells can be replaced by cancer cells, the so-called "cancer/tumor-on-a-chip, where TME can also be introduced in the model.

The tumor-TME organ-on-a-chip model allows tumor cells to interact with TME components at a short distance and under physiological conditions and can introduce a variety of TME cell types on one chip so that we can better explore the effect of the mutual interactions between tumor cells and TME components on tumor development, and drug resistance *in vitro* can be explored.

4.2. Establishment of TME in cancer-on-a-chip systems

4.2.1. CAF in cancer-on-a-chip models

In 2016, Jeong et al. (Jeong, Lee, Shin, Chung, & Kuh, 2016) reported a cancer-on-a-chip model in which human CRC spheroids and fibroblasts were co-cultured in a collagen matrix incorporated microfluidic chip. In this model, HT-29 cells-derived 3D tumor spheroids (TS) were cultured in the collagen-matrix supported channels and co-cultured with or without fibroblasts. Significantly low levels of doxorubicin uptake were observed in the co-cultured spheroids. Moreover, when exposed to paclitaxel, a survival advantage was observed in TS co-cultured with activated fibroblasts (Jeong et al., 2016). In the cancer-on-a-chip co-culture model of pancreatic cancer PANC-1 cells and pancreatic stellate cells established by Lee and colleagues (Lee et al., 2018), it was also observed that the mutual interactions of tumor cells and mesenchymal cells within a distance of 1 mm promoted the proliferation and epithelial-mesenchymal transition (EMT) of tumor cells, with the activation of mesenchymal cells. In addition, compared to single drug use, the combination of paclitaxel and gemcitabine produced a synergistic effect on tumor cell suppression, which was attributed to the selective inhibitory effect of paclitaxel on the viability of pancreatic stellate cells. That model proved useful in the evaluation of pharmacological effects by determining the differential sensitivity of different cell types and optimizing the drug combinations. The observed effect of CAFs on tumor resistance is consistent with a previous report (Amornsupak, Insawang, Thuwajit, Eccles, & Thuwajit, 2014), which proves the effectiveness of organ-on-a-chip platform in recapitulating the mutual interactions between tumor cells and TME components. Moreover, the organ-on-a-chip platform has great advantages in real-time monitoring and imaging compared with other models. Chung's group has established a 3D microfluidic cancer-on-a-chip platform to co-culture different types of cancer cells with CAFs (Chung, Ahn, Son, Kim, & Jeon, 2017). By using live cell imaging, the authors found that ovarian, gastric, and CRC cells showed different morphological changes when co-cultured with CAFs.

4.2.2. Immune microenvironment in cancer-on-a-chip

The immune cell is a significant part of TME, which plays an important role in tumor development and drug resistance. Increasingly efforts have been made to address the following challenges: 1) Introduce the immune components on cancer-on-a-chip that can better recapitulate the complex tumor immune microenvironment (TIME); 2) Understand the immune suppression/activation mechanisms; 3) Develop new therapeutic strategies for tumors.

Pavesi et al. (Pavesi et al., 2017) have established a simple and customizable microfluidic chip model to study the ability of human TCR-engineered T cells in overcoming the physical and metabolic barriers of the tumor. In that model, liver cancer cells were incorporated into the microfluidic devices in a 3D collagen gel area, and human T cells engineered to express tumor-specific TCRs (TCR-T cells) were then added to the adjacent channels (Pavesi et al., 2017). Based on the microfluidic chips, a new 3D cell co-culture model was established, which can be used to dissect the factors modulating the functions of T cells in solid tumors, and find application as preclinical models for developing personalized immunotherapy.

In another study (Lee et al., 2018), researchers co-cultured human primary monocytes with liver cancer cells on a 3D static microfluidic model and observed that monocytes suppressed the cytotoxicity of retrovirally transduced (Tdx) TCR-T cells, but not mRNA electroporation (EP) TCR-T cells. Importantly, when co-cultured in 2D, the cytotoxicity of TCR-T cells engineered by both Tdx and EP toward cancer cells were not suppressed by monocytes, indicating that the 3D cancer-on-a-chip model was superior in predicting the efficacy of TCR-T cells when compared to standard 2D models (Lee, Adriani, et al., 2018).

Parlato and colleagues established a 3D microfluidic platform to study the interaction of SW620 cells and interferon-α-conditioned dendritic cells (IFN-DCs) (Parlato et al., 2017). The proposed platform was composed of a central culture chamber (immune chamber) and two flanking narrow chambers (tumor chambers). In the immune chamber, floating IFN-DCs were evenly distributed and showed non-adhesive movements, similar to the peripheral immune cells; in the tumor

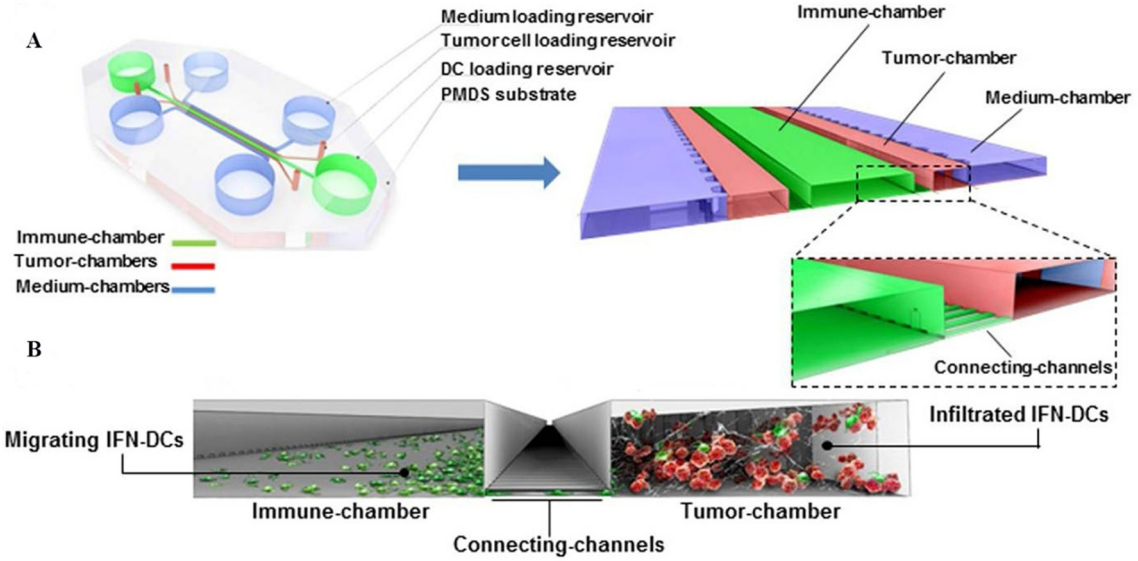


Fig. 3. The structure of a 3D immune-cancer microfluidic model. A) This microfluidic device consists of a central immune-chamber and two side tumor-chambers, which are connected through microgrooves (connecting-channels). Both ends of the tumor chamber and the immune chamber are closed with rounded loading reservoirs. There are also two external culture medium chambers on the outside of the tumor chamber for providing gas and nutrients to tumor cells. B) The DCs migrate from the immune chamber, through the connecting channel, to the tumor chamber and target tumor cells. Reproduced with permission (Parlato et al., 2017).

chambers, SW620 cells were cultured within the 3D type I collagen matrices (Fig. 3). Next, they treated one of the tumor chambers with histone deacetylase inhibitor romidepsin and IFN-α (RI SW620). It was found that IFN-DCs moved toward RI-treated cancer cells rather than NT counterparts. Moreover, IFN-DCs located near the RI space sensed tumor-specific stimuli and moved at a higher speed (Parlato et al., 2017). Then, it was found that SW620 cells treated with RI produced a large amount of CXCL12, while the expression of CXCR4 on DC cells was up-regulated, and the CXCR4/CXCL12 axis guided the movement of IFN-DCs to the RI space to capture apoptotic cancer cells. That platform allowed us to dissect the interaction between IFN-DCs and cancer cells in 3D spaces and helped uncovered its underlying regulating factors, such as the participation of CXCR4. Moreover, it emphasized its potential as an innovative tool for evaluating the efficacy of immunotherapy.

Jenkins et al. used a 3D microfluidic device to culture mouse-derived CRC organotypic TS together with autologous stromal and immune cells in collagen hydrogels for 3D culture *in vitro* (Jenkins et al., 2018). The reported system is compatible with microscopy and live/dead cell analysis, and dynamic multiple cytokine secretion analysis from conditioned media can be performed.

The efficacy of tumor immunotherapy largely depends on the tumor and TME heterogeneity of the individual patient. Around 80% of patients showed no response or side effects to immunotherapy (Topalian et al., 2012). As an *in vitro* 3D model, the above platform combining microfluidic/chip platform and TIME simulated the mutual interactions and influences between tumor and immune cells successfully, being useful as a preclinical prediction model for tumor immunotherapy. However, the co-cultured immune cell type is quite limited for the moment and still cannot fully simulate the complex TIME in the body. The future development trend is introducing more immune components (*e.g.*, including T cells, B cells, macrophages, and DC cells) into the co-culture system with tumor cells to explore the impact of their interactions on tumor progression and evaluate the overall immunotherapy efficacy.

4.2.3. Vasculature microenvironment in cancer-on-a-chip

Blood vessels are a strong component of TME, which provide oxygen and nutrients for tumor growth, but constitute the path for cancer cells metastasis(LaGory & Giaccia, 2016; Potente, Gerhardt, & Carmeliet, 2011). Angiogenesis inhibitors are important parts of targeted therapy for the treatment of cancer, but the response of tumors to anti-angiogenic therapy is complex and uncertain (Rivera & Bergers, 2015). Therefore, simulating the interaction between the tumor and its surrounding blood vessels *in vitro* can provide a better understanding of tumor progression and metastasis, and it can also provide an *in vitro* model for evaluating the efficacy of angiogenesis inhibitors.

In 2019, Nguyen et al. established a PDAC-on-a-chip model (Nguyen et al., 2019), which was composed of two hollow cylindrical channels and completely embedded in 3D collagen matrix (Fig. 4). In one channel, they seeded endothelial cells to form a biomimetic blood vessel. In the parallel channel, they seeded primary mouse pancreatic cancer cells PD7591 and allowed them to adhere to form a monolayer of epithelial cells to mimic the pancreatic duct. It was observed that the epithelial cells kept in contact with each other, forming a branch structure, showing a collective invasion as depicted in Fig. 4. Upon contact with the biomimetic blood vessel, the PDAC cells wrapped around the blood vessel and spread along the length of the blood vessel before invading the vessel itself, where the PDAC tumor cells invade the blood vessels and ablate the endothelial cells, leaving behind tumor-lined and tumor-filled luminal structures. The phenomenon of vascular ablation observed in the microfluidic model is consistent with that observed in the *in vivo* model later (Nguyen et al., 2019). By means of introducing the vascular components into the PDAC-on-a-chip model, the apparent hypovascularity of PDAC observed was not a result of reduced angiogenesis *per se*, but instead a consequence of endothelial ablation triggered by the invading tumor cells, giving us a deeper understanding of the role of TME in tumor progression.

Carvalho et al. also established a similar co-culture model with CRC cells and vascular endothelial cells on the microfluidic chip to evaluate precise nano-drug delivery (Carvalho et al., 2019). HCT-116 cells were seeded in the middle chamber embedded in Matrigel® supplemented with vascular endothelial growth factor (VEGF). In its turn, human colonic microvascular endothelial cells (HCoMECs) were seeded in the lateral channels around the middle chamber. It was found that HCoMECs sprouted to the middle chamber along the VEGF concentration gradient. Once the model was established, gemcitabine-loaded nanoparticles were injected into the lateral channel to simulate tumor chemotherapy. Results showed that the drug release concentration gradient was similar

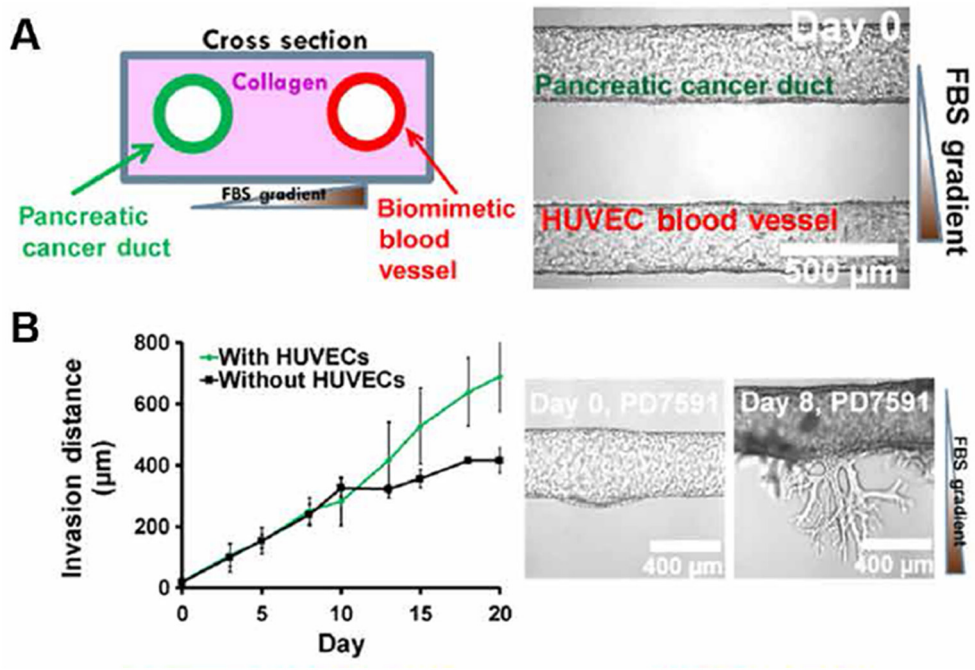


Fig. 4. The hollow cylindrical structure of the PDAC-on-a-chip model enables cancer cells and endothelial cells to form a 3D lumen. A) In the PDAC-on-a-chip model, endothelial cells form a biomimetic blood duct in one channel, and pancreatic cancer cells were seeded in another channel to form a pancreatic cancer duct. B) The invasion speed of pancreatic cancer cells toward a gradient of FBS was increased in the presence of human vascular endothelial cells (HUVECs) compared to the absence of HUVECs. Reproduced with permission (Nguyen et al., 2019).

to that in solid tumors. Through real-time monitoring of the cell changes with video images and transcriptome analysis of the Matrigel®/cell mixture of the middle chamber obtained with biopsy forceps, they found that only about 40% of cell death could be observed even after 5 days of exposure to the maximum drug concentration in this model (Carvalho et al., 2019). Th low level of cell death observed is consistent with a previous report, confirming the fact that 3D cultures usually show reduced sensitivity to anticancer drugs (Wen et al., 2013). The above results allow people to observe tumor chemotherapy responses similar to that *in vivo* through 3D models and reduce unnecessary late-stage animal and clinical experiments which may be followed by false positive results of the 2D model. That innovative cancer-on-a-chip platform is a promising tool, which simulates the drug concentration gradient in solid tumors through the vascular system. Cells from the chip can be easily extracted and drug dose-response can be studied *via* measuring the gene expression and monitoring the high-content images, and valuable information about mechanisms of drug action could be obtained. In addition, patient-derived cells (PDCs) can be used for personalized drug screening (Carvalho et al., 2019). Such a type of 3D platform is more suitable than previous models for evaluating the efficacy/toxicity of drugs in a more physiological environment.

At present, the vascular endothelium in most tumor vascular models is derived from HUVECs. To reflect the real situation *in vivo*, organ-specific vascular endothelium cells or separate vascular endothelium cells directly obtained from primary tumor lesions should be explored in the design of 3D models for future studies.

5. Organoid-on-a-chip platforms

The cancer-on-a-chip model facilitated achieving a real-time observation and precise regulation of the state of tumor cells, but the tumor cells used in these models are often derived from cell lines, which are still inferior to tumor organoid models in terms of tumor heterogeneity, phenotypic fidelity, and physiological complexity. Therefore, researchers began to explore the possibilities of culturing tumor organoids on the microfluidic chip platform called organoid-on-a-chip, to avoid their respective shortcomings and combine their advantages.

5.1. Engineering organoid-on-a-chip

Organoid-on-a-chip technology is being explored as a platform to fulfill the needs and major technical challenges in organoid research, due to some inherent obstacles. One of the main obstacles is the control of nutrient supply. Organoids depend on passive diffusion to receive nutrients and oxygen. The larger they grew, the tougher it was to meet the requirement of metabolism (Park, Georgescu, & Huh, 2019). The ability of organoid-on-a-chip to mimic vascular perfusion could tackle the issue of limited nutrient supply. The control of biophysical/biochemical cues is also presented as a challenge. Specific mechanical forces (biomechanical cues) and molecules (growth factors and signaling cues) are challenges in constructing a physiologically relevant model (Park et al., 2019).

In 2018, by combining stem cells with micro-engineering technology, Wang et al. presented a new approach for engineering liver organoids derived from hiPSCs in a 3D chip system (Wang et al., 2018). They established this model by sequentially adding growth factors to differentiate hiPSCs into liver organoids in a short time and maintain the long-term culture of the organoids in the chip system. Compared with in static culture, the liver organoids showed higher cell activity, higher levels of albumin secretion, and liver enzyme activity in perfusion culture on the chip, indicating that the organoids in the dynamic culture system exhibited improved hepatic-specific function (Wang et al., 2018). In general, during the long-term culture period, the liver organoids produced on the chip retained the tissue morphology and liver function for up to 1 month (Wang et al., 2018). Therefore, this 3D organoid-on-a-chip culture system provided a physiological microenvironment that integrates cell-cell interactions and mechanical fluid flow, which promoted the differentiation of liver organoids and the preservation of liver-specific functions.

In addition, Nikolaev et al. designed a hybrid microchip system for intestinal organoid culture derived from intestinal stem cells (Nikolaev

et al., 2020). The reported system consisted of an elastic device with a central cavity for hydrogel loading and organoid culture, and a pair of (inlet and outlet) reservoirs on both sides for cell loading and intracavitary perfusion. The microfluidic middle chamber is made into a shape similar to the natural structure of the small intestine and the side chamber uses hydrogel as the basis to provide culture medium and growth factors. That system allowed us to successfully establish an organoid cell tube similar to the natural intestinal cavity in the middle chamber. The lumen, open on both sides, can be used for liquid lavage and removing non-mucosa and dead cells in the organoid tube (Fig. 5) (Nikolaev et al., 2020). For the conventional organoids, dead cells shed and filled the closed cavity of organoids after 6–10 h without perfusion, leading to organoid destruction after a few days. When fresh culture medium or even growth factor free medium was injected into the lumen every 12 h to remove dead cells from the organoid tube, the lifespan of the organoid could be prolonged to one month or more, thus establishing a stable organoid culture system in which organoid grew for a long time without passage (Nikolaev et al., 2020). The diversity of cell types in steady-state intestinal organoids cultured on this chip system is similar to that of intestinal epithelium *in vivo*, including rare or non-existent cell types in conventional organoids, and maintains intestinal secretion and absorption functions. This is a self-sustainable organoid culture platform in which frequent passage was avoided, even some rare cell types could be maintained, and the intestinal tube can regenerate and retain the structure and function of the intestinal tract as much as possible. The biomimetic 3D ECM surrounds the small intestinal epithelium in this system, allowing easy planting of non-epithelial cell types such as endothelial cells, immune cells, and myofibroblasts, as well as intestinal microorganisms and parasites into the lumen of intestinal epithelium (Nikolaev et al., 2020). Such a highly biomimetic and dynamic 3D model *in vitro* allows us to investigate the effects of various carcinogenic factors on the pathogenesis of digestive cancers, and develop more new preventive and treatment strategies for digestive system tumors.

Another interesting organoid-on-a-chip model consists of culturing the primary duodenal epithelial organoids derived from biopsies with primary human intestinal microvascular endothelial cells (HIMECs) in two parallel cell culture microchannels on the chip. Each microchannel has a dedicated inlet and outlet for inoculation of human cells, molecules, or microorganisms, and precise control of physical and chemical parameters by perfusion of laminar flow of medium (Kasendra et al., 2018). Waste water from each chamber was collected from the outlet for downstream testing. This special-shaped chip enables the intestinal organoids to form a physiological intestinal cavity. The special stretchable chip material allows intestinal organoids to simulate intestinal peristalsis *in vitro*. Compared with organoids cultured alone on a chip, in the presence of primary HIMECs, organoid fragments can fuse together in a shorter time. This organoid-on-a-chip model presents the important structural features and functions of the natural duodenum, including the formation of elongated villi-like structures lined by a polarized epithelium expressing markers of enterocytes, goblet cells, enteroendocrine cells, and Paneth cells, as well as basal proliferating cells, exerts better barrier function, digestive function and secretive function, and all these functions are superior to ordinary intestinal chips and organoids. The organoid-on-a-chip system can dynamically collect and analyze the biochemical compounds produced or secreted by intestinal epithelial cells or endothelial cells, such as inflammatory cytokines or metabolites, which is impossible to achieve by conventional organoid culture. Vascular endothelial microchannels (which do not exist in conventional organoid cultures) can be opted to analyze the absorption of nutrients or drugs and drug bioavailability, as well as to characterize physiologically relevant pharmacokinetic parameters (Kasendra et al., 2018).

5.2. Organoid-on-a-chip for drug screening and precision medicine

The complex organoid-on-a-chip platform has powerful advantages for drug screening. Schuster et al. reported a high-throughput 3D organoid-on-chip culture and analysis system in 2020 (Schuster et al., 2020). This platform consists of two integrated devices, a 3D culture chamber device, and a multiplexer fluid control device. Interestingly, it is also equipped with custom software for automated and programmable experimental control, and live-cell time-lapse fluorescence microscopy. The two-layer culture chamber chip consists of a 200-well array chamber and an upper fluid channel (Fig. 6A-C). The array chamber is divided into 20 different subsets of 10 independent chamber units, which can accommodate up to 10 different patient-derived samples and implement 20 different treatments for parallel testing (Fig. 6D), and when the 3D organoid culture is exposed to a predetermined protocol, they can be simultaneously imaged in 3D *via* phase contrast and fluorescence deconvolution microscopy to provide real-time measurements of organoid reactions, movements, and proliferations (Fig. 6E, F). The programmable microscope is also equipped with an environmental chamber (incubator) for continuous temperature and humidity control. At the end of the experiment, the upper fluid supply channel can be removed, the lower cell-containing array chamber can be exposed, and the 3D cultures/organoids are possible to be harvested for subsequent analysis (sequencing, and amplification.) (Schuster et al., 2020). Through personalized, combined, and sequential drug screening on human-derived pancreatic cancer organoids, the authors reported significant differences in the response of individual PDOs to drug treatment and found that temporally-modified drug treatments can be more effective than constant-dose monotherapy or combination therapy *in vitro*. This platform integrates tumor organoid models and automated high-throughput organ-on-a-chip models, recapitulates the response of patients to personalized drug therapy *in vitro*, and proves to be a high-throughput, highly efficient, automated, and accurate anti-cancer drug screening platform, which may be used for anti-cancer drug screening and personalized precision therapy.

6. The future of complex *in vitro* 3D models

Although the above-mentioned complex *in vitro* 3D models have demonstrated their powerful application values, the current models still have plenty of room for improvement. Firstly, to simulate the complete TME, more components should be introduced into the tumor models. These components include the ECM, blood vessels, immune cells, and even microorganisms (pyloric spiral, bacillus, intestinal flora, and parasites) that reside in the digestive tract. Encouragingly, relevant studies have been tried in the digestive system. For example, Rajasekar reported in 2020 that a 384-well microfluidic platform named "IFlowPlate" can be used to culture up to 128 independently perfused and vascularized colon organoids *in vitro* (Fig. 7) (Rajasekar et al., 2020). Since vascular endothelial cells cannot self-organize into functional vessels in traditional Matrigel®, they mixed 10% v/v Matrigel® with fibrin gel and proved that human primary endothelial cells that mixed with human fibroblasts self-assembled into a microvascular network within three days. Then, the stem cells derived from intestinal tissues *in vitro* were implanted into the gel matrix to form organoids. Images demonstrated that the organoids were tightly surrounded by vessels, and both organoids and microvascular networks showed hollow cavities which could be observed from the cross-sections of the vascularized organoids. In addition, the vascular structure is not static and changed every day. The self-assembled vascular network continuously adjusted its structure according to the state of the flow and the nearby organoids, which is a valuable physiological feature. This platform can be used to culture colon organoids for at least 13 days, and the entire vascularized colon organoids can be removed from the hole for further histological analysis (Rajasekar et al., 2020). After establishing this model, the ability of the model to simulate human diseases was analyzed. With this model, they found that under the induction of TGF, monocytes which were perfused into the lumen of the self-assembled vessels migrated across the endothelium and infiltrated into the organoids, differentiated into macrophages, and gathered around the

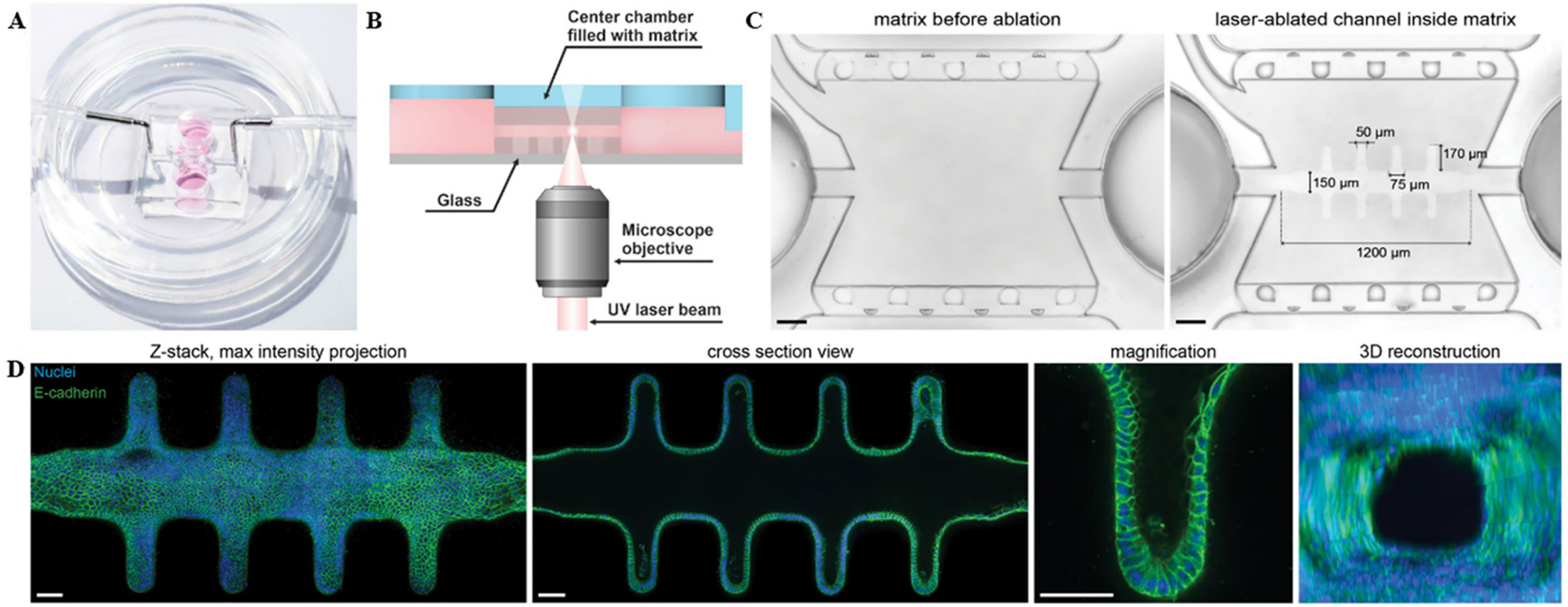


Fig. 5. Intestinal organoid-on-a-chip. A) The fully assembled microchip system. B) Laser ablation using a nanosecond-pulsed laser. C) Hybrid collagen type I/Matrigel® scaffold in the central chamber. D) Fluorescence images of the intestinal tube formed by organoids. Reproduced with permission (Nikolaev et al., 2020).

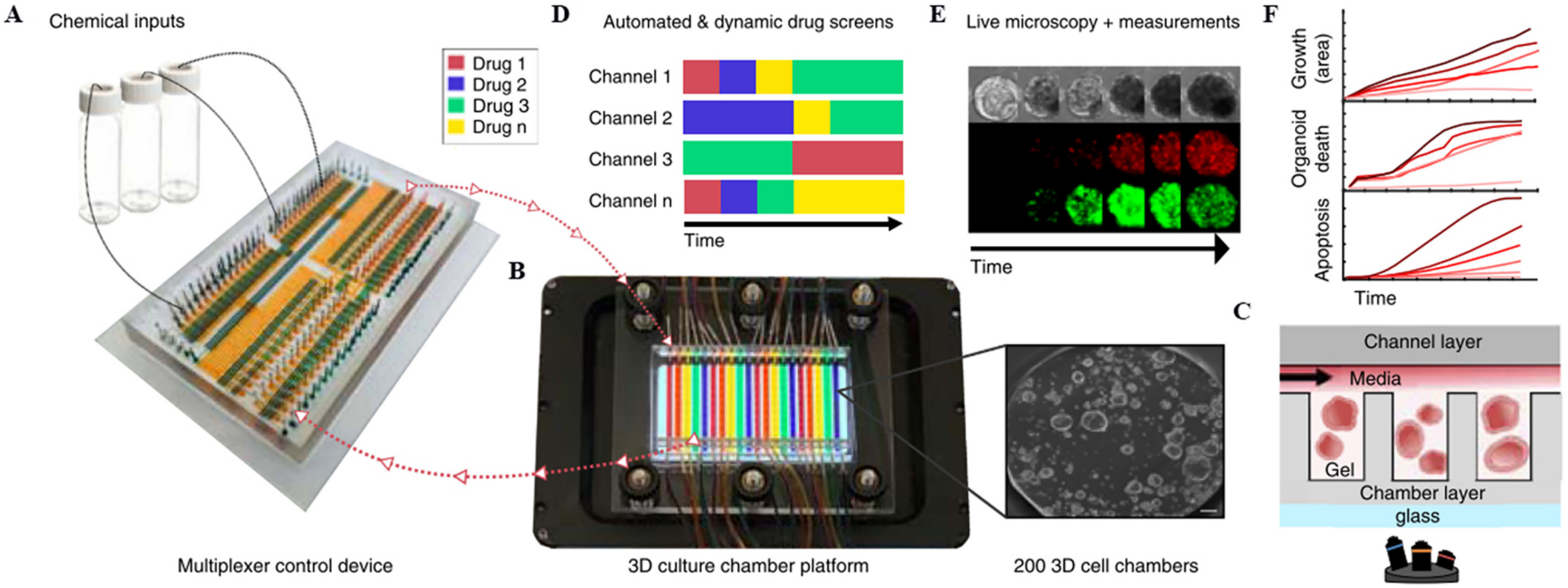


Fig. 6. A high-throughput 3D organoid microfluidic chip culture and analysis system for dynamic drug screening. A) The programmable control platform of the microfluidic chip provides automatic stimulation instructions for each chamber of the culture platform, resulting in many dynamic and parallel culture experiments. B) There are 200 individual chambers in the 3D culture chamber platform. C) Cross-section of two-layer 3D culture chamber based on polydimethylsiloxane. D) Device (A) is pre-programmed to provide stimulation with multiple drug combinations to Device (B). E, F) Time-lapse imaging of organoids is used to quantify drug stimulation results, and the 3D culture chamber device can also be disassembled to harvest cells for further experiments. Reproduced with permission (Schuster et al., 2020).

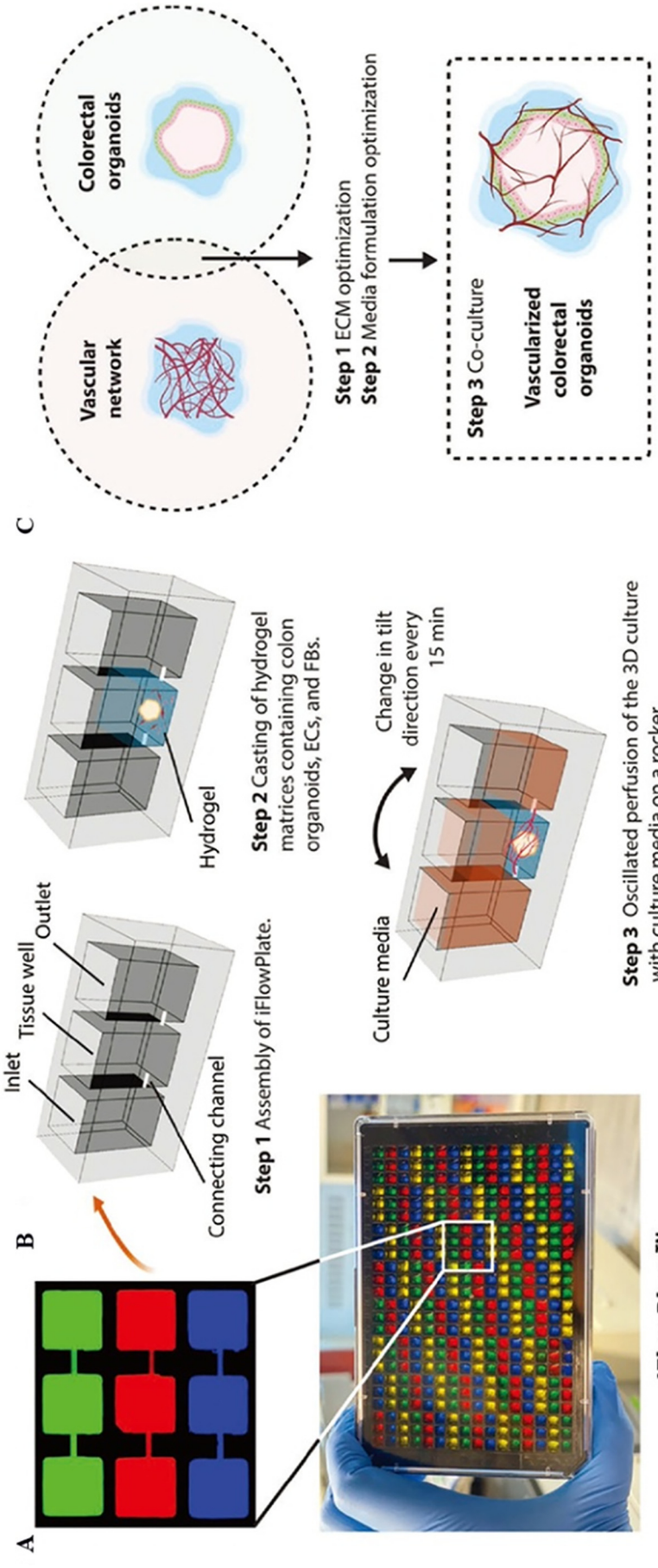


Fig. 7. Operation and setup of IFlowPlate. A) 128 independent units (each unit consists of three wells of the inlet, tissue, and outlet) depicted in different colors constitute the IFlowPlate. B, C) On the bottom of the tissue well, matrices containing colon organoids, endothelial cells, and fibroblasts are poured. The medium is perfused into the vascular system controlled by a programmable rocker. Reproduced with permission (Rajasekar et al., 2020).

organoid fragments (Rajasekar et al., 2020). The observed phenomenon indicated that the new model faithfully recapitulated the process of colon inflammation *in vitro*. The model is believed to be able to simulate the process of tumor immune infiltration when used in tumor organoids-on-chips.

Secondly, an important limitation to be considered is that the current models are still limited to a single organ-derived organoid-on-a-chip model. Multi-organoids-on-a-chip model, which integrates multiple organs derived organoids into one chip, can maximize the strength of organoids and organ-on-chips, and provide an effective tool to study the role of TME on tumor multi-organ metastases and evaluate the therapeutic effects of drugs. The multi-organoids-on-a-chip can simulate the whole process of absorption, distribution, metabolism, and excretion of drugs *in vivo*. For example, we can try to integrate CRC organoids, liver, and lung organoids into a chip model to simulate the process of liver and lung metastasis of CRC, or integrate digestive system tumor organoids, liver, and kidney organoids for drug screening. On the one hand, we can screen for effective anti-tumor drugs, on the other hand, the hepato-renal toxicity of anti-tumor drugs can also be monitored.

Thirdly, with the development of engineering and the integration of multidiscipline including biology, engineering, micro-electromechanics, material science, automation, and information technology, the organoid-on-a-chip model will soon reach a higher level, *i.e.*, the goal of short-time modeling, high-throughput, and automated monitoring and testing will be quickly achieved. For example, through equipping with microscopes, micro-fluorescence, mechanical measurement, multiple electrode arrays, and other analysis systems, the organoid-on-a-chip system can be modified into a high-throughput multiplexed sensing system and used for drug and phenotypic screening. By means of introducing a high-density silicon microelectrode sensing system into the organ-on-a-chip system, researchers analyzed the light-sensitive cells in brain organoids and demonstrated that the combining of high-throughput reading sensors with organoids is beneficial for studying complex cell interactions (Quadrato et al., 2017).

Finally, with the development of high-throughput technology in biology, especially in oncology, researchers have obtained massive biology data in a short time. They combined these data with analytical computer-based technologies to build a highly sophisticated computational *in silico* models, that aid in oncology research, drug development, and personalized medicine (Edelman, Eddy, & Price, 2010; Sacan, Ekins, & Kortagere, 2012). For example, Ma et al. developed a model for predicting the efficacy of anti-pancreatic cancer drugs and identified seven drugs, three of which were supported by the literature results, and three were verified by *in vitro* experiments (Ma et al., 2016). The *in silico* model is complementary to traditional *in vitro* and *in vivo* modeling. Therefore, combining the organoid and microfluidic/chip models with *in silico* approaches can precisely predict the efficacy and toxic side effects of drugs and minimize costly preclinical experiments (Zietek, Boomgaarden, & Rath, 2021). Lewin et al. constructed a microfluidic model of infiltration of T cells into intestinal mucosa, which recapitulates T cell bispecific antibody (TCB)-mediated T-cells activation and epithelial cells killing (Lewin et al., 2022). They analyzed the imaging data of the situation of T cell infiltration observed in wet experiments with *in silico* model to explore the dynamic changes of T cell infiltration in the presence of TCB. It holds great promise in predicting the efficacy and safety of novel cancer immunotherapy drugs by combining *in silico* and microfluidic/chip models.

7. Conclusions

Digestive system cancers impose a significant economic burden on our healthcare system. Therefore, it is important to better understand the pathogenesis and development of digestive system cancers and screen for personalized cancer therapies. Cancer research relies heavily on the application of cell culture and animal models. It is well known that different tumor and TME cell subgroups play different and important roles in determining the ultimate therapeutic effect. The stronger the ability of a model to preserve intratumoral heterogeneity and mimic the physiological environment (*e.g.*, gastrointestinal tract lumen, fluid pressure, mechanical stress, concentration gradient of biochemical substances) of parental tumors, the more accurate it will be in reproducing tumor progression and predicting patient drug responsiveness. In this review, we discussed the PDOs, cancer-on-a-chip, and organoids-on-a-chip systems that incorporate parental tumor heterogeneity and tumor-TME interactions in a high-throughput fashion. These complex *in vitro* 3D models have good application prospects in precision medicine, particularly in identifying new targets/mechanisms and predicting patient-specific responses. For example, for tumor patients with multidrug resistance, the engineered PDO models can conduct high-throughput screening on a variety of chemotherapy drug combinations in a short time to guide clinical treatment. Of course, more powerful parallel clinical trials are still needed to prove the accuracy of predictions.

As described above, the ideal 3D model should be able to recapitulate the *in vivo* microenvironment, including integration of multiple cell components, *in vivo* physiologic and biochemical environment, and exchange of material and information between different cells and tenvironment. Currently, the organoid-on-a-chip model seems to be closer to this goal. The future organoid-on-a-chip model of digestive system tumor should contain digestive system tumor organoids and all or most of the TME components (*e.g.*, ECM, mesenchymal cells, immune cells, micro-vessels, nerves, and microorganisms). Organoids and cell components should preferably come from tumor patients to represent the heterogeneity of the tumor. The chip system precisely controls the biochemical (*e.g.*, O_2, CO_2, nutrients, and growth factors) and physiologic (*e.g.*, fluid pressure, electrical stimulation, and GI peristalsis) environment to form a controllable, repeatable, and easy-to-manipulate cell environment, and can be used to automatically monitors and collects data in real-time. Individualization and standardization should be taken into account when the chip model was put into practice, the inter- and intra-tumor heterogeneity determines the personalized medication, while standardized operating procedures can help increase repeatability and decrease the error of experimental results caused by differences in operating and culture conditions, completely or partially replace animal experiments, and truly realize precision medicine and efficient drug testing.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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