



Gastric Organoids: Progress and Remaining Challenges

Min-Jiao Pang,¹ Joseph R. Burclaff,² Ramon Jin,³ Mahliyah Adkins-Threats,³ Luciana H. Osaki,³ Yunan Han,⁴ Jason C. Mills,^{3,5,6} Zhi-Feng Miao,¹ and Zhen-Ning Wang¹

¹Department of Surgical Oncology and General Surgery, Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, Ministry of Education, The First Affiliated Hospital of China Medical University, Urumqi, China; ²Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ³Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine in St. Louis, St. Louis, Missouri; ⁴Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine in St. Louis, St. Louis, Missouri; ⁵Department of Pathology and Immunology, Washington University School of Medicine in St. Louis, St. Louis, Missouri; and ⁶Department of Developmental Biology, Washington University School of Medicine in St. Louis, St. Louis, Missouri

SUMMARY

We introduce the differing methods for culturing healthy gastric tissue from adult tissues, pluripotent stem cells or gastric cancer tissue. We also discuss the promise these systems have for preclinical drug screens and highlight the applications of organoids for precision medicine.

The stomach is a complex and physiologically necessary organ, yet large differences in physiology between mouse and human stomachs have impeded translation of physiological discoveries and drug screens performed using murine gastric tissues. Gastric cancer (GC) is a global health threat, with a high mortality rate and limited treatment options. The heterogeneous nature of GC makes it poorly suited for current “one size fits all” standard treatments. In this review, we discuss the rapidly evolving field of gastric organoids, with a focus on studies expanding cultures from primary human tissues and describing the benefits of mouse organoid models. We introduce the differing methods for culturing healthy gastric tissue from adult tissues or pluripotent stem cells, discuss the promise these systems have for preclinical drug screens, and highlight applications of organoids for precision medicine. Finally, we discuss the limitations of these models and look to the future to present potential ways gastric organoids will advance treatment options for patients with GC. (*Cell Mol Gastroenterol Hepatol* 2022;13:19–33; <https://doi.org/10.1016/j.jcmgh.2021.09.005>)

Keywords: Stomach; Organoids; Gastric Cancer; Tumor Micro-environment; Preclinical Trial.

The mammalian stomach is lined by an ordered epithelium consisting of invaginated gastric units housing varied cell types (mucous pit cells, acid-secreting parietal cells, zymogenic chief cells, and proliferative and intermediate populations).¹ Research into gastric epithelial biology has long been hindered by lack of accurate models, as gastric tissue is historically difficult to culture and there are notable physiological differences between mouse and

human stomachs.² Recent advances in our ability to culture 3-dimensional (3D) self-renewing organoids from mouse and human stomachs have opened many possibilities for studying gastric cells.^{3–5} In the past decade, researchers have used gastric organoids to better probe basic stomach biology, identify cell plasticity, analyze interactions between the gastric epithelium and immune cells or pathogens such as *Helicobacter pylori*, and gain valuable insights into the progression and treatment of gastric cancer (GC).^{6–9}

GC is a major public health issue, ranking as the fifth most common malignancy and the fourth-leading cause of cancer-related deaths worldwide. One study attributed about 770,000 global deaths to GC in 2020 alone.¹⁰ Therefore, it is necessary to improve GC treatments both in terms of efficacy as well as safety. GC prognosis is poor, with surgery currently the only curative option.¹¹ However, surgery alone is oftentimes not enough and multimodal treatments including incorporation of per-operative chemotherapy is now routine to increase survival rates.¹² For metastatic or advanced GC, systemic chemotherapy, targeted therapy, and immunotherapy are the only treatments. Current 2- to 3-drug chemotherapy regimens only modestly benefit overall survival, with median overall survival under 12 months.^{13,14} Another novel treatment is molecular targeted therapy, but there are only 2 targeted therapies currently approved and although many new therapeutics are tested every year, very few are validated clinically.¹⁵

One major hurdle for developing effective treatments for human cancers is the lack of accurate experimental platforms to identify new therapies and to test efficacy of

Abbreviations used in this paper: 2D, 2-dimensional; 3D, 3-dimensional; ALI, air-liquid interface; CagA, cytotoxin-associated gene A; DC, dendritic cell; EGF, epidermal growth factor; ESC, embryonic stem cell; FGF10, fibroblast growth factor 10; GC, gastric cancer; ORISC, organ-restricted adult stem cell; PD-L1, programmed death ligand 1; PDO, patient-derived organoid; PDX, patient-derived tumor xenograft; PSC, pluripotent stem cell.

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therapies on individual patients to personalize their treatment. Animals are useful research models for some aspects of developing anticancer therapy, such as testing safety and efficacy of new regimens, yet many drugs that clear pre-clinical animal trials fail during clinical trials due to differences in animal and human physiology.^{16,17} As an alternative, organoids have recently become a popular *in vitro* culture model for developmental biology, drug screening, and disease modeling.¹⁸ Organoids can be developed from human and animal cells and tissues, recapitulate more of the cellular complexity of actual tissues, and are a common preclinical model.^{16,17} As well as their use in the field of cancer, gastric organoids have shed light on stomach development and progressed our knowledge of pathogenic infection and immune response in the stomach. Here, we describe the application of gastric organoids to the study of basic stomach biology and disease states and elaborate on their potential for implementation in clinical practice as a guide for precision medicine.

Gastric Organoid Culture

Organoids are stem cell-originated, self-organized 3D clusters of organ-specific cells capable of maintaining aspects of the functionality and molecular and cellular heterogeneity of the originating organ. Organoids have been cultured from many tissues including the brain, retina, kidney, liver, and intestine.^{19–23} However, even with the inherent self-organization capacity of stem cells, elaborate physiologically relevant tissues cannot be formed in all conditions. Rather, the experimental environment is paramount in steering cellular development in a highly context-dependent manner.²⁴

Organoids can be derived from 2 sources of stem cells: organ-restricted adult stem cells (ORISCs) and pluripotent stem cells (PSCs),^{24,25} both in the form of induced PSCs and embryonic stem cells (ESCs) (Figure 1, Table 1). PSC-derived organoids are obtained by mimicking the sequential signaling interactions operating during *in vivo* development, whereas organoids derived from ORISCs are obtained by replicating signaling cues native to the respective adult tissues.²⁴ Compared with ORISCs, PSCs possess a broader potency that allows for directed differentiation into organoids resembling many adult tissues, and their increased potency also allows for coordinated generation of cells from multiple germ layers. Both fundic and antral gastric organoids have been developed from PSCs by the Wells group.⁴ These PSC-derived organoids do not require a biopsy and comprise diverse populations of gastric epithelial cells (like functional parietal and chief cells) and a surrounding layer of undifferentiated mesenchymal cells.⁴ On the other hand, ORISCs are epithelial derived, and do not contain components of the tissue microenvironment, highlighting their restricted potential compared with PSCs. Nonetheless, ORISC-based organoids faithfully recapitulate homeostatic conditions and regenerative processes of the adult tissue.²⁶

Ultimately, a major application of *in vitro* organoid cultures is for the study of the *in vivo* stem cell niche.

Simulation of these niche signaling cues in an *in vitro* culture system allows stem cells to proliferate and differentiate into tissue-specific cell types. Human gastric organoids are commonly cultured by seeding gastric glands from human gastric resection tissue in a basement matrix and culturing in a medium containing epidermal growth factor (EGF), R-spondin-1, Noggin, Wnt-3a, fibroblast growth factor 10 (FGF10), gastrin, an inhibitor of ALK5/4/7 (aka TGFBR1, ACVR1B, or ACVR1C), the small molecule inhibitor A83-01, and SB202190.²⁷ Using these growth conditions, gastric glands can grow into gastric organoids.^{28,29} Alternate methods exist which rely on co-culturing organoids with stromal elements rather than extrinsically added growth factors such as the air-liquid interface (ALI) technique (Figure 2).³⁰ In the following section, we will describe technical aspects of gastric organoid modeling and their derivation from cells that have stem-like characteristics.

Organoids From Organ-Restricted Adult Stem Cells

ORISC-derived gastric organoids are developed from primary human stomach tissues. Multiple strategies have been used to enable long-term growth of stomach tissue into organoids structures. One protocol uses a collagen type I gel (Trevigen, Gaithersburg, MD) with an ALI to support the growth of organoid epithelial structures.³¹ Organoids can be grown with fetal calf serum alone, but growth is improved by supplementation with R-spondin1, similar to ALI intestinal organoids.³⁰

A second protocol relies on distinct growth factors and extracellular support provided by laminin-rich Matrigel to support epithelial growth.³² Notably, Bartfeld et al²⁹ used this method, based on a protocol developed earlier for culture of mouse antrum,³³ to generate gastric organoids from human antral/pyloric stomach tissue. They isolated gastric glands from human gastric corpus tissue then seeded them in Matrigel with media supplemented with various growth factors (Wnt-3a, R-Spondin-01, Noggin, N2, B27, FGF10, EGF, gastrin, nicotinamide, etc.). Organoids can be generated from the gastric cardia and expanded similarly under the same culture conditions. Matrigel is used to provide a suitable environment for the embedded gastric glands and to provide extracellular support. After seeding, human gastric glands seal and form small cysts that subsequently expand.

Organoids From PSCs

Gastric organoids derived from ORISCs have limitations. Establishing gastric organoids from ORISCs requires access to human tissue samples, which is not commonly available to many laboratories.⁴ Even when available, the quality of these tissue samples is widely variable and heavily dependent on timely processing.⁴ Moreover, ORISC-derived organoids are also further limited for use in cancer studies due to their lack of microenvironment. An alternate approach is to generate gastric organoids from human PSCs, which include both human ESCs and induced PSCs. The unique ability of PSCs to both self-renew and differentiate into cell

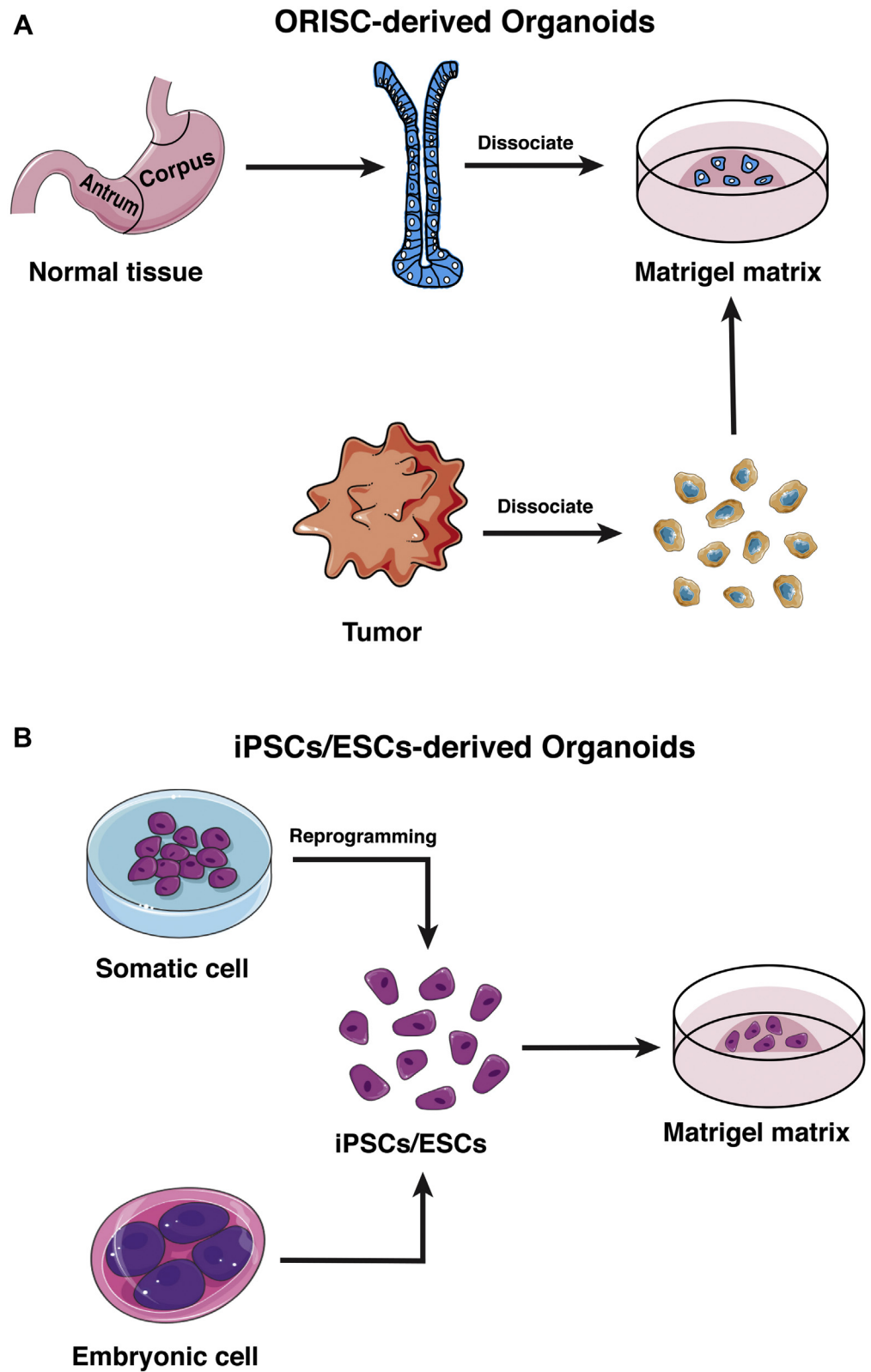


Figure 1. An overview of current approaches to develop gastric organoids *in vitro*. Gastric organoids can be generated from 2 sources of stem cells: ORISCs and PSCs. ORISCs are mainly derived from human gastric tissues samples such as endoscopic biopsy specimens. Gastric gland cells or cancerous cells were collected and plated into the Matrigel matrix. PSCs include both induced pluripotent stem cells (iPSCs) and ESCs; iPSCs can be derived from reprogrammed adult somatic cells or blastocysts. Typically, isolated ORISCs or PSCs were embedded into Matrigel matrix domes and cultured with media supplemented with necessary growth factors.

Table 1. Characteristics of 2 Different Sources of Organoids

Feature	PSC-Derived Organoids	ORISC-Derived Organoids
Pluripotency	Yes	Limited
Time needed	6–8 wk	1 wk
High-throughput screening	Yes	Yes
Modeling human disease	Yes	Yes
Modeling organogenesis	Yes	Limited
Precision medicine	Limited	Yes

ORISC, organ-restricted adult stem cell; PSC, pluripotent stem cell.

types from multiple lineages permits modeling the whole organogenesis process in cell culture.²⁴

Recently, Noguchi et al⁵ used a method to differentiate mouse ESCs into organoids consisting of gastrointestinal endoderm surrounded by mesoderm. However, this approach used mouse ESCs aggregation and spontaneous differentiation, resulting in heterogeneous organoids.⁷ Moreover, stomach morphology and regionalization differ greatly across species,^{34,35} making animal models like the mouse stomach suboptimal for simulating the major structure and physiology of the human stomach.^{7,29,36,37} For example, the largest volume of the postprandial mouse stomach is composed of the forestomach, a nonglandular squamous epithelium similar to the esophagus. The forestomach is not present in humans.²⁹ Thus, to effectively study human gastric development, physiology, and disease, it is imperative to use a human model system.

McCracken et al³⁸ identified a step-wise differentiation approach to generate human gastric organoids, whereby PSCs were sequentially differentiated into definitive endoderm, patterned to posterior foregut, then specified into a pure antral epithelium with normal antral cell types. They began by differentiating PSCs into definitive endoderm by adding activin A, a transforming growth factor β family member that stimulates the highly conserved Nodal signaling pathway required for endoderm formation across vertebrate species.³⁹ Endoderm was then patterned into anterior and foregut endoderm by inhibiting BMP signaling with Noggin. Foregut spheroids were directed into posterior foregut by activation of the retinoic acid signaling pathway. Wnt activation promoted the development of human fundic gastric organoids, whereas simultaneous MEK inhibition and activation of the BMP signaling pathway promoted the differentiation of acid-secreting parietal cells that could be stimulated by histamine.^{4,7} These gastric organoids represent the first human antrum fully derived in vitro that recapitulates many of the most important aspects of stomach physiology.³⁸ So far, PSC-derived gastric organoids have been used as an in vitro system to identify signaling mechanisms that regulate human stomach development and physiology, and to model the pathophysiological response of

the gastric epithelium to *Helicobacter pylori* infection.^{7,38,40,41}

Organoids From Gastric Tumors

To distinguish organoids derived from tumor tissue from those derived from normal organoids, tumor organoids are often called tumoroids or patient-derived organoids (PDOs). A common culture protocol for culturing PDOs follows steps similar to those used for ORISC-derived organoids.²⁹ In short, tumor tissue is mechanically disrupted and enzymatically digested, then seeded in Matrigel and supplied with a certain mixture of growth factors, finally leading to PDO formation.²⁹ The ALI method has also been used to propagate PDOs by embedding the minced tissues inside a collagen gel with an ALI to support the growth of organoids epithelial structures.³¹ Different from normal organoids, the ALI method allows combined culture of epithelial and mesenchymal/stromal components like native immune cells (T, B, natural killer, macrophages) to develop together with the PDOs,^{31,42} using a technology already applied to intestinal organoids.^{43,44} Moreover, unlike normal PDOs, ALI-cultured PDOs can grow without A83-01, FGF10, and Wnt3a.¹¹

One obstacle to obtaining pure PDO cultures is that patient-derived nonmalignant organoids commonly develop from healthy cells within the tumor samples.^{19,45–48} Despite increased cell division in tumor-derived organoids, the nontumor organoids can eventually overgrow the tumor-derived organoids.⁴⁵ The reason for this seemingly paradoxical competitive advantage is not clear, but it may be due to a higher rate of mitotic failure and subsequent cell death in tumor cells.¹⁹ Another factor involved may be that seemingly normal cells near a tumor may also harbor many cryptic mutations that confer faster growth than truly normal tissue from a patient without precancerous or cancerous lesions.^{49,50} To circumvent these issues, multiple strategies have been developed to eliminate contaminating normal organoids. The first approach targets mutational patterns dictating growth factor dependency of the organoid culture (Figure 3).⁴⁵ Many GCs exhibit p53 pathway mutations,⁵¹ and this feature can be selected for using the small molecule Nutlin-3, an MDM2 inhibitor, which stabilizes TP53 by disrupting the binding of TP53 to its negative regulator E3 ubiquitin ligase, MDM2.⁴⁵ Notably, ROCK inhibition plays a significant role in the recovery of individualized normal organoid cells. Second, an additional strategy used has been to culture PDOs in a ROCK inhibitor (Y-27632)-free medium to enrich for RHO-dysregulated GC organoids.⁵² Third, organoids isolated from tumors harboring EGF receptor signaling pathway mutations can be selected for by EGF withdrawal or EGF receptor inhibition, which leads to loss of the patient-derived nonmalignant organoids.⁴⁵ Furthermore, if a culture displays a clear mix of populations, the normal organoids can be simply removed by phenotype-based manual selection.⁴⁵ Finally, clonal PDOs can be established from single cells collected via flow-cytometry based cell sorting.⁴⁵ However, all of these approaches may lessen cellular heterogeneity compared with

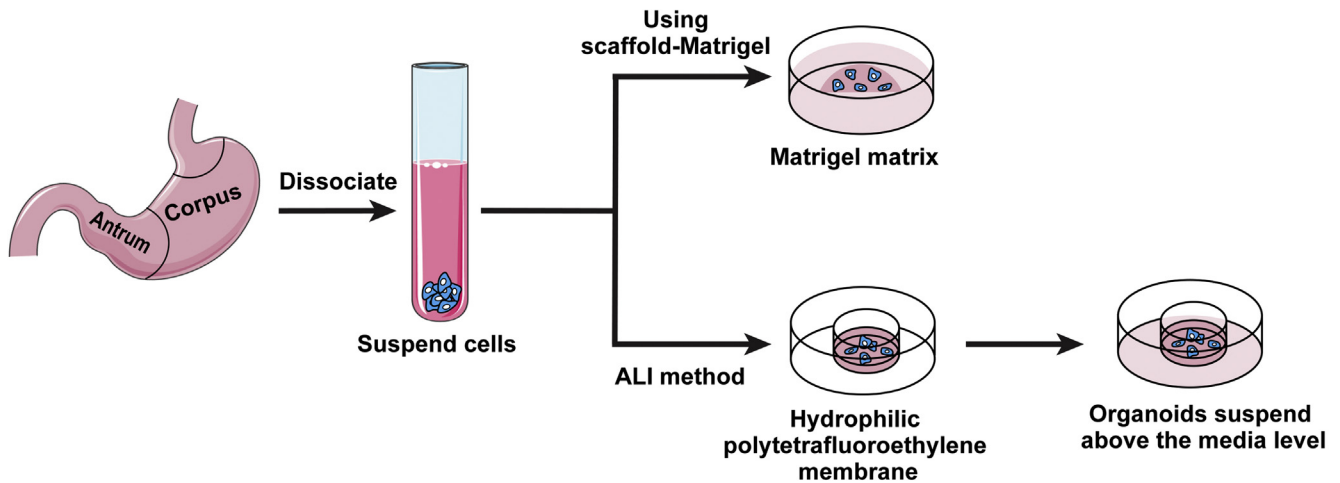


Figure 2. Differences between scaffold-Matrigel and ALI methods to generate gastric organoids. Gastric organoids can be generated from both scaffold-Matrigel and ALI methods. The scaffold-Matrigel culture system is a common and effective method to establish gastric organoids. First, isolated cells are mixed with Matrigel, the Matrigel is then allowed to polymerize, then culture medium supplied with various grow factors is added. For the ALI method, cells are placed onto a hydrophilic polytetrafluoroethylene membrane at the bottom with collagen solution. Different from the scaffold-Matrigel method, the top layer of the cells is directly exposed to the air instead of submerged in culture media, with only the basal surface keeping contact with the liquid culture media.

the initial cultures, so it is recommended that early passages of established cultures be frozen to serve as references for initial cell content.⁴⁵

Another consideration for PDO cultures is source tissue quantity. While surgical resection supplies an abundance of tissue for initiating organoid cultures, surgery is invasive to patients and many metastatic or advanced GC patients never undergo resection. Therefore, esophagogastroduodenoscopy is ideal for obtaining tissues for organoid creation, especially for advanced patients lacking good treatment options and for whom testing potential treatments on PDOs is most beneficial. To address this, Gao et al⁵³ innovated a new method to develop gastric tumoroids from endoscopic biopsies of patients with gastric adenocarcinoma. They found that a single endoscopic biopsy of GC can generate organoids that are reflective of the overall primary tumor and may be used for patient-related testing.⁵³ As collection of GC tissues via endoscopy poses minimal risks to patients, it may be a plausible way to culture tissue from metastatic or advanced GC patients who would never undergo resection.

Modeling Disease in Human/Mouse Organoids

Chronic *H. pylori* infection is one of the single most critical factors increasing risk for GC worldwide. Long-term *H. pylori* infection in the stomach in many individuals causes a multistep histopathological cascade known as the Correa pathway which ultimately leads to GC.⁵⁴ The use of gastric organoids has enabled many important discoveries regarding *H. pylori* pathogenesis. For example, only a small percentage of people infected with *H. pylori* will develop GC,⁵⁵ likely owing to genetic factors and/or specific interactions between host, microbial, and environmental

determinants.⁵⁶ Strains of *H. pylori* that produce the virulence factor cytotoxin-associated gene A (CagA) substantially increase stomach cancer risk compared with strains lacking CagA. Binding of CagA to the ASPP2 (apoptosis-stimulating protein of p53-2) causes mislocalization of PAR members, predisposing the infected cells to lose their cell polarity and gain an EMT-like phenotype promoted by the interaction of CagA with Par1b.⁵⁷ Human gastric organoids were used to show that a Cherry-tagged CagA-binding ASPP2 peptide could act as a sponge to reduce the CagA-induced phenotypes, abrogating the loss of cell polarity and reducing *H. pylori* colonization.⁵⁷

Dendritic cells (DCs) in the human gastric mucosa are thought to be the major antigen-presenting cells that induce protective immune responses to *H. pylori* infection. Recently, Sebrell et al. developed an in vitro co-culture model by adding human monocyte-derived DCs isolated from peripheral blood mononuclear cells to organoid cultures.⁵⁸ They found that bidirectional crosstalk between gastric mucosal DCs and epithelial cells that were infected with *H. pylori* by microinjection contributed to the maintenance of gastric homeostasis and found that DC recruitment to the gastric epithelium is driven mostly by CXCL1, CCL20, and possibly CXCL8 following *H. pylori* infection.⁵⁸

Another unique finding from in vitro human studies is that pathogenic *H. pylori* infection induces gastric epithelial cells to express programmed death ligand 1 (PD-L1), an immune checkpoint ligand known to suppress the immune system by shutting down T cell effector function.⁵⁹ Holokai et al⁵⁹ found that gastric organoids or monolayers derived from PSCs or adult tissues could survive chronic inflammation by expressing the immunosuppressive ligand PD-L1 throughout the infection and progression to cancer. This signifies that once a patient progresses to a metaplastic

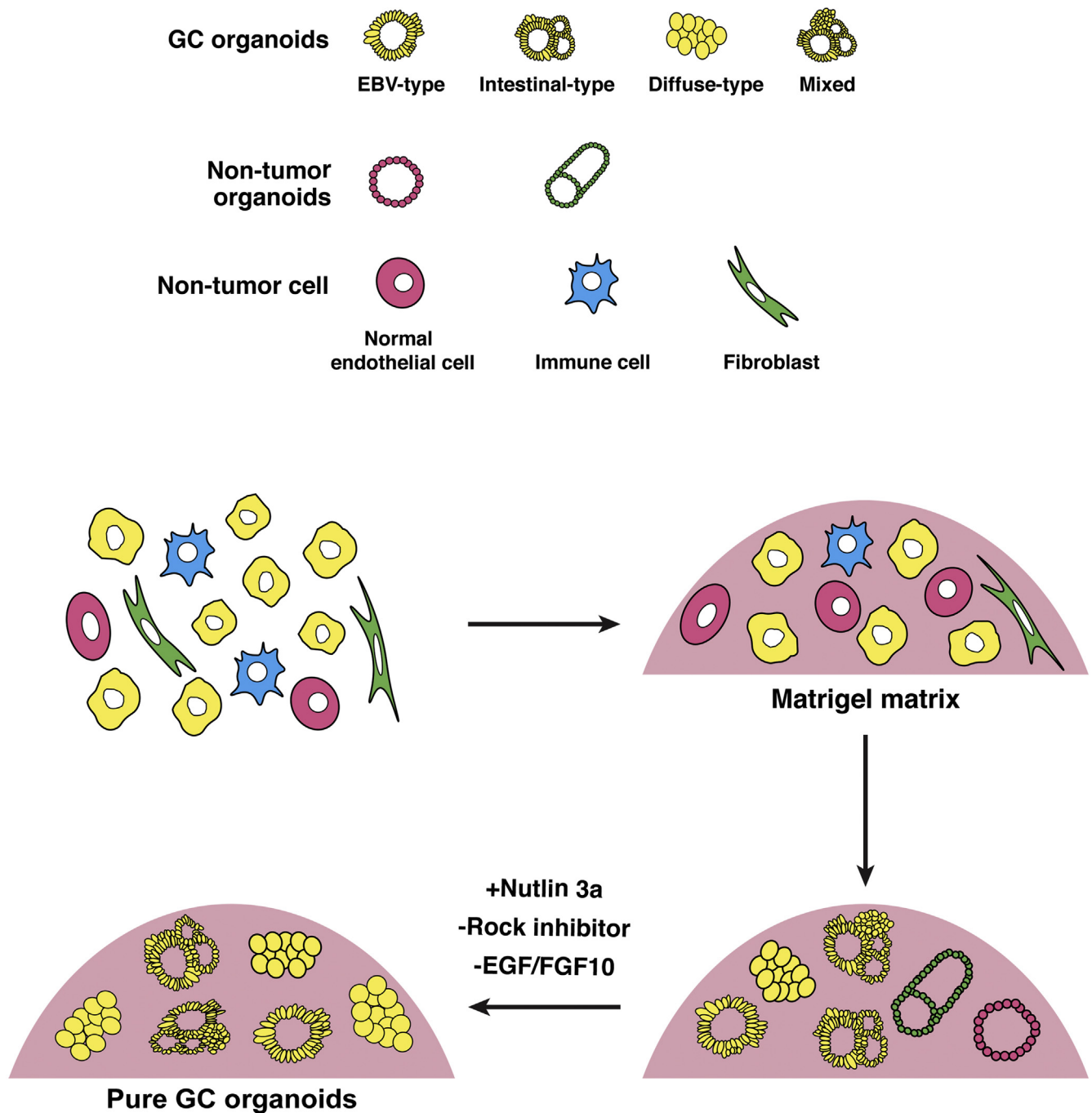


Figure 3. Establishing pure GC organoids. As primary tumor tissues often contain nonmalignant cells, nontumor organoids are commonly seen in patient-derived GC organoid cultures and eventually outgrow the tumor organoids. Therefore, it is essential to establish pure GC organoids for specific experiments like drug screening. Removing or adding certain growth factors in the culture medium allows for establishing pure GC organoids derived from various kinds of lesions such as primary tumors, metastases, and carcinomatous ascites with noncancerous tissues eventually lost.

state, eradication of *H. pylori* may not decrease the risk of developing GC.⁶⁰ Furthermore, a meta-analysis showed that PD-L1 expression lasts through GC development, and up to 69% of all GC expresses PD-L1.⁶¹

The use of murine gastric organoids has gained popularity in recent years as well. While these murine models do not fully replicate human stomach physiology or genetics,

mouse models and organoids derived from mouse stomach mucosa have become a vital tool for studying mechanisms of tumorigenesis. Mouse gastric organoids are generally cultured in a Matrigel matrix with growth factors similar to those described for human culture.³³ Dedicated progenitor cells within adult tissues have been considered the main candidate cells of origin for cancer,⁸ yet recent work

suggests that mature differentiated cells may contribute as much or more to cancer initiation in the stomach and other organs, especially in organs without dedicated progenitor stem cells like the pancreas and liver.^{62,63} In fact, the idea that cancers could arise via recruitment of differentiated cells had been one of the prevailing theories prior to the dominance of the stem cell theory in the latter half of the 20th century.⁶⁴ Lineage tracing reporter mice, as well as tracing of cells that incorporate DNA analogs, have shown that multiple cells are capable of exhibiting progenitor activity in the stomach in both homeostatic and metaplastic (ie, precancerous) states. Gastric corpus organoids have been shown to be able to originate from normal epithelial progenitors or from proliferating chief cells.^{65,66} For example, lineage tracing using a chief cell-specific marker, Troy, validated the existence of multipotent progenitor cells at the gland base in the gastric corpus. Sorted Troy⁺ chief cells were able to form long-lived organoids that can differentiate toward the mucus-producing cell lineages of the neck and pit region.⁶⁵ The cellular process by which mature chief cells re-enter the cell cycle in response to tissue damage was found to be regulated by mTORC1 and conserved among other cell types from a variety of differing organs.⁶⁷ This process has been termed paligenosis.⁶⁸ Loss of key genes regulating paligenosis such as *Ifrd1/Ddit4* can result in growth changes in corpus organoids, indicating that paligenotic chief cells contribute to organoid outgrowth.⁶⁹

The ability to genetically manipulate mouse models allows for more specific studies on disease initiation and progression. Dysplasia is a key transition state between precancer and cancer in gastric tumorigenesis.⁷⁰ Min et al⁷¹ derived gastric organoids from a *Mist1-Kras(G12D)* mouse model and examined functional roles for *Kras* activation in the progression of dysplasia by inhibiting MEK, a downstream mediator of *Kras* signaling. *Kras* activation was found to control cellular dynamics and progression to dysplasia, and dysplastic stem cells appeared to contribute to cellular heterogeneity in dysplastic cell lineages.⁷¹ Similarly, another group found that *Kras*^{G12D} expression or p53 loss cause gastric organoids to develop dysplasia and easily generate adenocarcinoma upon *in vivo* transplantation.⁷² Furthermore, TFF1-knockout mouse-derived gastric organoids readily led to a proinflammatory phenotype with a cascade of gastric lesions that include low-grade dysplasia, high-grade dysplasia, and adenocarcinomas, indicating that gastric tumorigenesis may be suppressed by TFF1 impeding the IL6-STAT3 proinflammatory oncogenic signaling axis.⁷³

Mouse organoids have also been well used to better understand mechanisms of disease development, especially with regards to *H. pylori* pathogenesis.⁷⁴ Morey et al⁴¹ used organoids from both mouse and humans to demonstrate that *H. pylori* expression of *cgt* (the first in a series of *H. pylori* enzymes) reduced cholesterol levels in infected gastric epithelial cells, thereby blocking interferon gamma signaling to allow the bacteria to escape the host inflammatory response. The Zavros group has also made extensive use of mouse organoids as a model to analyze how gastric tissue responds to *H. pylori* infections, notably implicating

the gene CD44 as promoting the increased proliferation following infection.⁷⁵ Through these varied uses, mouse gastric organoids have greatly increased our knowledge of the gastric epithelial response to injury and disease.

Personalized Medicine for GC Patients Using Pdos

With the advent of next-generation sequencing, single-cell RNA sequencing, and novel preclinical modeling strategies, GC research is undergoing a radical shift toward precision medicine.⁷⁶ PDOs comprise effective tools for genetic evolution studies, biomarker identification, drug screening, and preclinical evaluation of personalized medicine strategies for GC patients. Subsequently, we discuss how PDOs have contributed to current GC research and discuss their future possibilities.

Drug Screening

Standard 2-dimensional (2D)-cultured human cell lines (in other words, standard tissue culture cell lines predominantly isolated from malignant cells and cultured on plastic) have been particularly important in drug studies, and have been the primary source for studying drug responsiveness to identify novel drug targets for decades. Indeed, results from cell culture studies are a significant factor in deciding whether or not a drug should advance beyond preclinical trials in humans.^{77,78} However, the high failure rate of new drugs that show efficacy and activity in these traditional preclinical studies has substantially increased the associated costs of drug development, demonstrating the need for more representative models of human organ systems for drug screening during the preclinical phase.⁷⁸ Moreover, 2D cell lines are prone to genotypic drift and cross-contamination, may fail to establish permanent cell lines after long-term culture, and can exhibit loss of tumor heterogeneity.⁷⁹ Therefore, organoids (especially from tumors) that lack tumor stroma and vasculature fall in between purely 2D cancer cell lines and patient-derived tumor xenografts (PDXs) (Table 2).⁷⁹ Although significant medical advances have been made using standard 2D cell culture models, these static models cannot effectively recapitulate the physiology of living tissues.⁹ In physiological conditions, cells reside in a complex environment constantly interacting with other cells and the extracellular matrix; and these interactions are critical for proper tissue differentiation and function. Organoid culturing techniques promote cell-to-cell interactions and can oftentimes more precisely mimic physiological and pathological conditions.⁸⁰ For example, a recent study using organoids derived from human breast cancer showed higher levels of reactive oxygen species production and increased resistance to cisplatin compared with standard 2D cell cultures.⁸¹ Importantly, organoids have been shown to exhibit different drug metabolism and secretion properties due to their different environmental cues, making them well suited for the study of drug therapies.⁸⁰ Because organoids more accurately represent human disease, they have tremendous potential for predicting *in vivo* drug sensitivity and responses.⁸²

Table 2. Comparison of Organoids With 2D Cell Lines and PDX Models

Feature	2D Cell Lines	Organoids	PDX
Heterogeneity	-	+	++
Genome editing	+++	++	-
High-throughput screening	+++	++	-
Modeling organ development/ disease	+	+++	++
Modeling tumor micro-environment	-	++	+

For the representation of each respective feature, +++ indicates the best, ++ indicates suitable, + indicates possible, and - indicates unsuitable. 2D, 2-dimensional; PDX, patient-derived tumor xenograft.

As PDOs retain the heterogeneity and histological characteristics of the primary tumor, they represent an ideal model for drug screening. Hence, establishing large PDO libraries function as living biobanks and combining with drug screening might be a powerful tool to delineate novel therapeutic strategies in GC generally.⁸³ Recently, Yan et al⁸⁴ established a primary GC organoids biobank encompassing normal, dysplastic, and cancerous stomach tissue as well as lymph node metastases from 34 patients. This unique GC organoid biobank encompassed nearly all known GC molecular subtypes and different stages of disease. The authors used this biobank to demonstrate that large-scale drug sensitivity screening is feasible.⁸⁴ Their organoid-based drug screen of 37 anticancer drugs was timely (taking <2 weeks), and, most importantly, was able to correlate with actual patient in vivo responses for several new targeted anticancer drugs including VE822, an ATR inhibitor.⁸⁴ These data suggest that organoid based pre-clinical testing may help guide future cancer therapeutic choices.

In addition to standard 2D cell cultures, the PDX model, in which patient cancer cells or tissue are implanted into immunodeficient mice to recapitulate the patient's tumor biology, is also an important preclinical model.⁸⁵ However, these PDX models have many inherent disadvantages including significant time and resource constraints in comparison to PDO models (Table 2). The organoid drug screening platform will likely be a more practical route to informing patient treatment and as a screening tool in clinical trials to accelerate anti-cancer drug development.⁸⁴ Moreover, GC organoid biobanks may serve as a useful tool for drug screening by bridging the gap between ex vivo and in vivo models by more accurately portraying the genetic profile of these cancers while decreasing time and resource costs.^{83,86} However, despite the numerous advantages of organoid culture, the various techniques used to propagate the cultures are still relatively new and need to be further improved to enhance drug response and testing.⁷⁸ Standardizing the procedures for initiating and propagating gastric tumor organoids would be beneficial in maintaining biological relevance and predictability across studies.⁸⁷ In the next section, we will discuss the use of PDOs to optimize personalized cancer treatments.

Precision Medicine

GC is a heterogeneous disease featuring many different histological and molecular subtypes. Specifically, GC can be categorized into 4 major genomic subtypes: microsatellite instability, Epstein-Barr virus, intestinal (chromosome instability), and diffuse (genomically stable).⁵¹ Each group is characterized by a distinct molecular profile of genes that are dysregulated, implicating unique therapeutic targets within that subgroup of GCs to be further evaluated in clinical trials. However, despite the pronounced interpatient and intratumor heterogeneity of GC and our further understanding of the molecular subtypes that make up this disease, cancer therapy approaches for GC have remained more or less homogeneous, with uniform treatment strategies used for virtually all patients.

With the advent of novel preclinical modeling strategies such as in vitro organoid cultures, GC treatment studies are increasingly focusing on precision medicine.⁷⁶ The goal of personalized or precision medicine is to tailor therapy to specific patients expressing a certain molecular abnormality to maximize efficacy and minimize side effects. If PDOs are going to help us realize the promise of personalize medicine, it will be critical to ensure that the organoids will mimic the intratumor and interpatient genetic heterogeneity.⁸⁸ Updated molecular genetic profiling of GC has yielded promising new therapeutic targets such as receptor tyrosine kinases, RAS, and PI3K signaling proteins.⁷⁶ Thus, the integration of GC genotype, phenotype, and drug sensitivity testing using PDOs models promises to accelerate the use of personalized anticancer therapy, to improve treatment outcomes, and to assist in future clinical trial design and personalized medicine strategies.^{26,89}

Outside classical chemotherapy, only 2 targeted treatments have been approved by the U.S. Food and Drug Administration to take advantage of genetic alterations as molecular targets for novel treatment options: the anti-HER2 agent trastuzumab and the antiangiogenesis agent ramucirumab.^{11,88,90-92} Thus, organoid lines may constitute innovative molecular subtype-specific model systems to test individualized treatment regimens.⁹⁰ GC organoids have already been used to help predict patient response to targeted therapies such as HER2 inhibition.⁸⁸ Steele et al⁸⁸ optimized culturing organoids in a 96-well-plate format for use in drug testing within 3 days of the patient's surgery,

making it is feasible to study molecular subtype, perform a drug screen and provide guidance on individualized adjuvant therapy for each patient within 5–6 days after surgery. Similarly, Vlachogiannis et al⁹³ cultured cancer-derived organoids from patients with gastrointestinal metastatic cancers and treated them with commonly used therapeutics to predict treatment response. They found 100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value for organoids forecasting the patient response to targeted agents or chemotherapy.⁹³ Importantly, they were able to mimic interpatient tumor differences using patient-derived organoids and also distinguish intra-patient tumor heterogeneity in response to chemotherapeutic drugs.⁹³ A third study showed that chemoradiation responses in patients were highly matched to rectal cancer organoid responses, with 84.43% accuracy, 78.01% sensitivity, and 91.97% specificity.⁹⁴ These studies indicate the strength of organoids to predict tumor-specific responses and potentially represent a first step toward personalized treatment regimens using PDOs.⁹⁵

As mentioned previously, Gao et al⁵³ published a novel technique for establishing GC organoids from endoscopic biopsies, which may yield clinically relevant results for patients who are ineligible for surgical intervention. Their technique yielded abundant esophagogastroduodenoscopy-derived organoids that could be tested with multiple standards of care drug regimens and combination therapies within 2 weeks,⁵³ providing a valuable model for predicting therapies for individual patients with advanced metastatic GC. PDOs cultured from diagnostic biopsy procedures may also allow for simultaneous testing of multiple drug regimens to guide therapy in a clinically relevant time interval, an important step towards personalized medicine for GC patients.⁹⁶ As nearly all potential GC patients undergo endoscopic or diagnostic biopsies during their treatment course, biopsy-initiated organoids may also allow for widespread biobanking of not only GC organoids from many different patients, but also GC organoids from the same patient during meaningful time intervals throughout their disease course.⁹⁶

The promise of *in vitro* organoid systems for personalized medicine also extends to analyzing or even modulating the patients' immune response to disease. Given the advent of checkpoint inhibitors and immunotherapy in frontline GC treatment,^{97–99} better models are needed to study and optimize these treatments. PDO models are unable to mimic the *in vivo* tumor microenvironment completely, as they normally lack blood vessels for studying cancer angiogenesis and metastasis, and also lack the immune component found within the native tumor environment. However, recent studies have demonstrated that co-cultures of organoids and immune cells can partially overcome this limitation. The ALI culture method can preserve the complex histological tumor microenvironment architecture with tumor parenchyma and stroma, including functional tumor-infiltrating lymphocytes.³¹ Moreover, this PDO system allows for *in vitro* modeling of tumor microenvironment-intrinsic immune cell responses as opposed to those driven by peripheral immune populations.³¹ Dijkstra et al¹⁰⁰ demonstrated that

co-culturing tumor organoids with autologous peripheral blood lymphocytes can enrich for tumor-reactive T cells from peripheral blood of patients with mismatch repair-deficient colorectal cancer and non-small cell lung cancer. These activated T cells can then kill tumor cells from the same patient. This provided proof of concept to generate a novel class of tumor-specific T cell products derived from the peripheral blood, and established a means to assess the sensitivity of tumor cells to T cell-mediated attack in individual patients.¹⁰⁰ Moreover, Zavros' group co-cultured transgenic mouse-derived gastric tumoroids with autologous immune cells specifically for the study of PD-L1/PD-1 interactions within the tumor microenvironment.¹⁰¹ Presently, the exploration of organoids co-cultured with immune cells is still in a relatively early stage, but these findings are significant and demonstrate that PDOs recapitulate many key aspects of cancer immunobiology such as upregulation of checkpoint proteins like PD-L1 that promote immune evasion and microbial persistence.^{61,102} These co-culture models may help optimize the response of effector T cells specifically against a patient's tumor or provide a means to generate large numbers of effector T cells targeted to tumor cells for potential adoptive cell transplantation.¹⁰³ While still early in development, these GC patient-specific model systems hold great promise for implementing personalized medicine and targeted therapy. It is clear that future advances in PDO GC models will allow for combining traditional genome- and phenotype-based strategies to rapidly advance precision medicine applications.⁷⁶

Future Perspectives and Limitations

Since the Clevers group discovered the combination of culture factors needed to maintain the division and differentiation of intestinal stem cells in a 3D environment a decade ago,³ the worldwide application of organoid technology has resulted in unprecedented advances for many organs and diseases, including the study of GC.

Owing to the heterogeneous nature of tumors, especially in GC, no drug can be effective for all patients. Thus, personalized medicine is needed to advance cancer therapy. Tumor-derived organoids are rapidly becoming an important tool to realize this goal. Drug screening using human primary cancer organoids can aid in developing personalized treatment strategies, and PDOs from early-stage human cancers may even help identify early molecular aberrations to be used as biomarkers and prevention targets.¹⁰³ Moreover, organoids can be generated from rounds of biopsies over time from cancer patients to continuously assess their treatment response, to detect any developing drug resistance, and to prospectively predict their response to future therapy options.¹⁰⁴ In 2019, Clevers' group identified that tumor organoids can be used to establish individualized *ex vivo* model systems to support T cell-based therapies and to study the interactions between T cells and tumor cells.^{100,105} They were able to test for tumor cell sensitivity and resistance to immunotherapy, potentially allowing for future unbiased generation of patient-specific T cell products.

Further incorporation and integration of microenvironment components will enable gastric organoids and tumoroids to more faithfully represent *in vivo* physiology. Currently, there are many disparate and evolving techniques using organoids to study epithelial and niche interactions. A thorough discussion of this topic is beyond the scope of this review and is covered in detail elsewhere.¹⁰¹ Specifically, microenvironment components may be added individually to gastrointestinal epithelial organoids such as immune components,^{95,101,104} mesenchymal cells,^{106,107} and even neurons.¹⁰⁸ Developmentally based co-culturing techniques have also been developed using PSCs that enables differentiation of the mesenchyme together with gastric epithelial organoids.³⁶ Finally, the ALI technique developed by the Kuo Lab is an additional 3D gastrointestinal organoid culturing technique that incorporates multiple components of the microenvironment including mesenchymal and immune components.^{29,30,109} Much work is needed to optimize these co-culturing techniques and to fully elucidate the limits of this *in vitro* system.

Techniques for how to best recapitulate the stomach's physiology are also rapidly evolving. Human "organs-on-a-chip," a multichannel 3D microfluidic cell culture chip that simulates the activities, mechanical properties, and physiological responses of the organ and organ system, is emerging as a new direction for constructing organoids models with higher physiological relevance. While organoids rely heavily on spontaneous self-assembly to generate their organized tissue structure, organoids-on-a-chip provide an artificial bioengineered system to arrange cells to simulate tissue or organ physiology.¹¹⁰ In the near future, additional bioengineering approaches such as live imaging, genome editing, and single-cell genomics may also be incorporated into these systems to better study human organogenesis, diseases and personalized medicine,¹¹⁰ possibly opening new avenues to advance this "next generation" of *ex vivo* organoid models.

Organoids are robust tools for studying human development and disease. However, it is important to note their limitations. Drawbacks to gastric organoids in general are difficult access to the lumen (require microinjection)²⁸ and the fact that organoids that are passaged over time *in vitro* lose the ability to differentiate into functional chief and parietal cells. Meanwhile, gastric organoid models help overcome the technical and biological restrictions of *in vivo* studies, but they lack nonepithelial cellular components including mesenchymal tissue, neural cells, and immune cells, as discussed previously. Another practical limitation for organoid cultures is the necessity of Matrigel or other animal-derived matrices in most organoid models to enable cells to aggregate into organoids.¹⁰⁴ The composition of these matrices is poorly defined, and their efficient removal is pivotal for subsequent DNA or RNA isolation, CRISPR/Cas9 editing, or cryopreservation.^{38,104} In addition, their use may preclude PDOs from truly being able to be integrated into CLIA-certified clinical applications. When studying tumors *ex vivo*, obtaining pure cancer organoids represents another crucial hurdle, as tumor organoids can be overgrown and contaminated by normal organoids derived from

healthy gastric epithelial cells intermixed in the starting tissue material.^{19,45–47,52} Investigators have begun to develop sophisticated means to molecularly select for tumor cells.^{52,84} While these limitations need to be noted and accounted for in experimental design, few are insurmountable. In addition, these limitations are also outbalanced by the many advantages of tumor organoid models such as their ease of use, benefits of their 3D physiology, and the ability to test tumor tissue from a patient in a time frame that allows for clinical decision making. It is clear that advancing organoid culturing techniques will improve our understanding of stomach physiology and pathology.

Conclusions

In summary, even though current organoids systems show limitations and require additional optimization for use in disease modeling and personalized medicine, they have opened up important new avenues for regenerative medicine and, in combination with additional bioengineering approaches, they will continue to be invaluable tools in preclinical and clinical research.

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Correspondence

Address correspondence to: Zhi-Feng Miao, MD, PhD, Department of Surgical Oncology and General Surgery, Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, Ministry of Education, The First Affiliated Hospital of China Medical University, 155 N. Nanjing Street, Shenyang, Liaoning Province, China, 110001. e-mail: zfmiao@cmu.edu.cn; fax: 8624-83283555. OR Zhen-Ning Wang, MD, PhD, Department of Surgical Oncology and General Surgery, Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, Ministry of Education, The First Affiliated Hospital of China Medical University, 155 N. Nanjing Street, Shenyang, Liaoning Province, China, 110001. e-mail: josieon826@sina.cn; fax: 8624-83283555.

Conflicts of interest

The authors disclose no conflicts.

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