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Review Article

What gastroenterologists and hepatologists should know about organoids in 2019

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ABSTRACT

Most of the research behind new medical advances is carried out using either animal models or cancer cells, which both have their disadvantage in particular with regard to medical applications such as personalized medicine and novel therapeutic approaches. However, recent advances in stem cell biology have enabled long-term culturing of organotypic intestinal or hepatic tissues derived from tissue resident or pluripotent stem cells. These 3D structures, denoted as organoids, represent a substantial advance in structural and functional complexity over traditional *in vitro* cell culture models that are often non-physiological and transformed. They can recapitulate the *in vivo* architecture, functionality and genetic signature of the corresponding tissue. The opportunity to model epithelial cell biology, epithelial turnover, barrier dynamics, immune-epithelial communication and host-microbe interaction more efficiently than previous culture systems, greatly enhance the translational potential of organotypic hepato-gastrointestinal culture systems. Thus there is increasing interest in using such cultured cells as a source for tissue engineering, regenerative medicine and personalized medicine. This review will highlight some of the established and also some exciting novel perspectives on organoids in the fields of gastroenterology and hepatology.

1. Organ in a dish

It has been extremely difficult to establish *in vitro* propagation of primary adult intestinal or liver epithelial cells without inducing genetic transformations. However, recent advances in stem cell biology have enabled the *in vitro* generation of complex three-dimensional multicellular stem cell-derived constructs that mimic their corresponding organ *in vivo* [1–3] (Fig. 1). These organ-like structures denoted as organoids (or organs in a dish) have been isolated from various vertebrate tissues, including human, mouse, cow, chicken and pig. Organoids have been successfully generated from many regions of the gastrointestinal tract, e.g. stomach, small intestine and colon as well as many other organs such as liver, gallbladder, pancreas, lung, mammary gland, prostate, ovaries, taste buds and lingual epithelium as well as the skin [4–22]. They can recapitulate the *in vivo* architecture, functionality and genetic signature of the tissue of origin. In fact, it has been demonstrated that organoids derived from the murine colon engrafted into dextran sodium sulfate (DSS) pretreated mice could regenerate normal crypts including all types of epithelial cells in the injured mucosal wall [23]. Thus, the organoid technology can be employed to reveal novel insights into basic biology such as stem cell biology, organogenesis, cellular differentiation, cell-cell interaction and physiological functions but also is important for the future of regenerative medicine. They can also be used to study the pathophysiology of various human diseases such as cancer, infection (host-microbe interaction), inflammation and hereditary diseases such as cystic fibrosis. Of note, organoids allow the field

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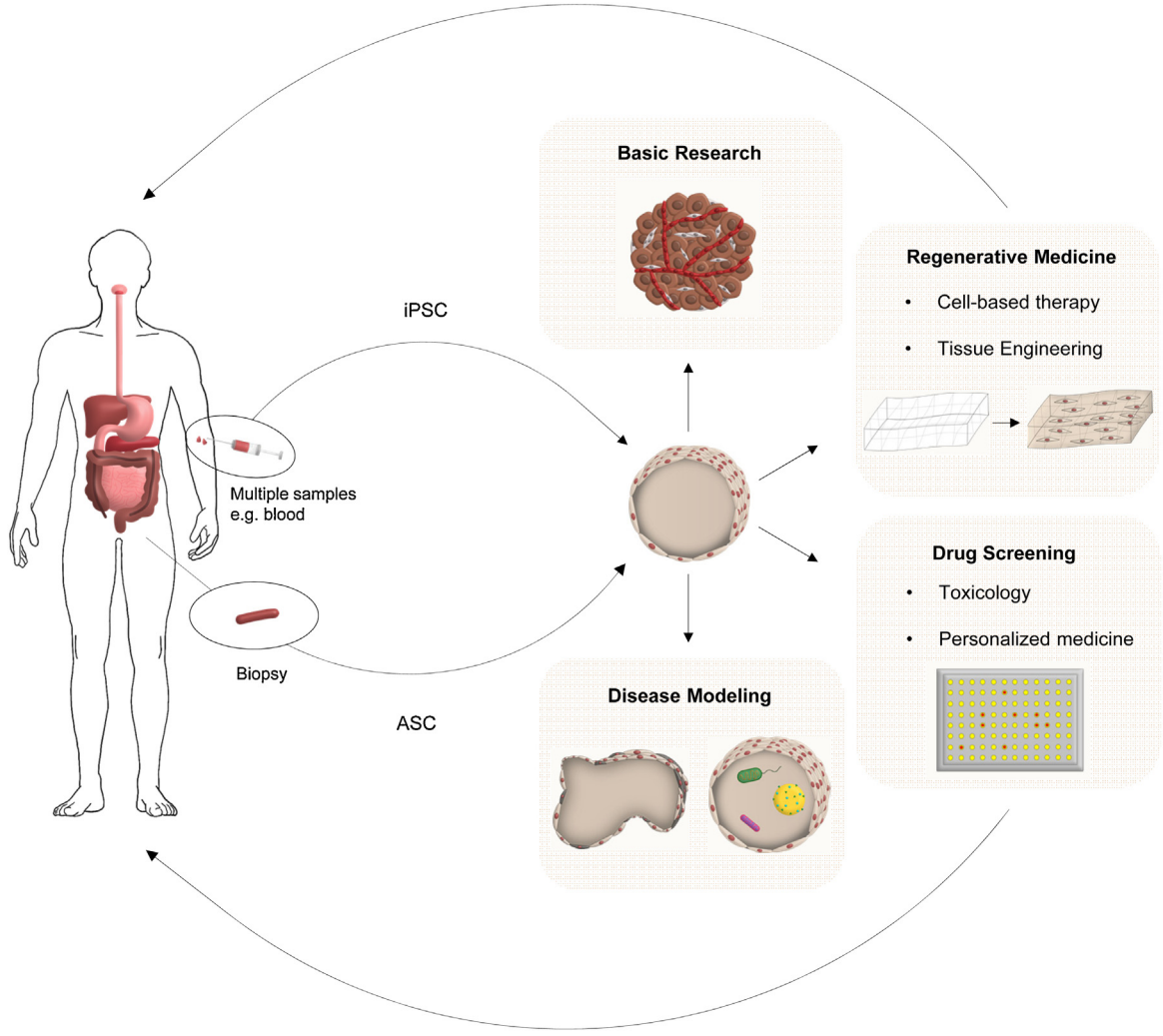

Fig. 1. Applications of organoids.
Organoids can be established directly from patient tissue samples, including fresh biopsies and resected material containing either organ-specific adult stem cells (ASC) or pluripotent cells (PSC), derived from skin or blood cells, reprogrammed and then differentiated towards the desired lineage. They faithfully recapitulate the genetic and epigenetic (primary tissue-derived organoids) landscape of the patient of origin providing unique opportunities for drug screening and personalized treatment strategies. Their capacity to engraft and survive *in vivo*; their ability to self-organize to complex structures resembling mini-organs *ex vivo*; and their potential to generate bioengineered tissue, makes them optimally suited for regenerative medicine. Furthermore, organoids can be used to model and study the pathophysiology of various human diseases such as cancer, infection (host-microbe interaction), inflammation and hereditary diseases. Since organoids serve as a novel tool to model epithelial cell biology, epithelial turnover, barrier dynamics, immune-epithelial communication and host-microbe interaction, organoids have been chosen the method of the year 2017 not only for medical approaches but also for basic science.

to move away from the often non-physiological, transformed cell lines that have been used in research for decades. Since organoids potentially serve as a novel tool in translational research to further explore human biology and diseases and their clinical application in regenerative therapy and personalized medicine, organoids have been chosen as method of the year 2017 by the journal Nature.

Organoids can be derived from two types of stem cells: either (1) pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) or (2) organ-specific adult stem cells (ASCs), which are tissue-specific resident stem cells [2,24–26] (Fig. 1). When grown in a 3D environment, these stem cells self-organize into organoids that replicate key structural and functional features of the corresponding *in vivo* organ. While organoids are very promising models to study human disease and to develop personalized medicine, they are far away from being perfect. Researchers are confronted with a variety of challenges such as the complexity of culturing organoids. Moreover, it is still not clear if and how these organoid cultures can recapitulate their corresponding tissue of origin *in vivo*.

The human gastrointestinal tract comprises the foregut, midgut and hindgut [27,28]. Each region gives rise to defined tissues and organs. Oral cavity, pharynx, esophagus, stomach, proximal duodenum and parts of the hepatobiliary system such as the liver parenchyma and pancreas develop from the foregut endoderm. The midgut and hindgut endoderm give rise to the other parts of the small intestine (distal duodenum, jejunum, ileum) as well as to colon, rectum, anal canal and also to the epithelium of the bladder and urethra. Although they share a common developmental origin, the physical turnover is highly variable between the different organs. This is highlighted by differences in generation and cultivation of organoids from these different tissues [29–32]. Maintenance and repair of adult tissues with high cellular turnover such as the gastrointestinal tract rely on small populations of actively cycling tissue resident stem cells. Various recent studies have impressively visualized these cells at the very bottom of the invaginations of the mucosa known as crypts in the small and large intestine and gland bottom in the stomach [29]. In sharp contrast, liver and pancreas represent organs with slow physical turnover [33]. Accordingly,

they do not contain a defined stem cell niche [34]. Adult homeostasis in the liver instead is mostly maintained by the self-replication of existing adult mature hepatocytes and ductal cells or in case of tissue injury or damage by resident progenitor cells.

2. Organoid cultures from the gastrointestinal tract (high turnover organs)

In 2009 the group of Prof. Clevers for the first time described the establishment of long-term culture conditions which enable single crypts, derived from the murine small intestine, to undergo multiple crypt fission events [35]. Simultaneously, the generation of villus-like epithelial domains containing all differentiated cell types takes place. The group introduced these structures as intestinal organoids and further demonstrated that a single Lgr5 positive intestinal stem cell is sufficient to give rise to these structures. Subsequently many other groups described similar protocols to isolate stem cell derived intestinal organoids [14,23,36]. They all have in common that they require a 3D extracellular matrix covered by medium supplemented with growth factors that resemble the *in vivo* stem cell niche environment. This medium mainly includes factors that stimulate the Wnt signaling pathway. Of note, Paneth cells which are only present in the crypt bottom of the small intestine are a rich source of WNT3A and thus are essential for small intestinal organoid cultures, whereas for colon organoids, WNT3A needs to be added ectopically [37,38].

Intestinal organoids can be derived either from the fetal or adult intestine [reviewed in [36,39]]. In contrast to intestinal organoids isolated from adult tissues, fetal epithelial organoids retain plasticity regarding their fate which reflects their immature state of development [40,41]. This is further highlighted by the morphological differences between these two types of organoids. While organoids derived from the small intestine of adult mice are characterized by several buds that represent the crypt region containing stems cells surrounded by Paneth cells, fetal organoids maintain a spheroid structure without invaginations [40]. Interestingly the tendency of forming spheroids decreases when organoids are isolated around postnatal day 15 [41]. This fact nicely recapitulates the developmental nature of the murine gut, since postnatal development of the gut, including Paneth cell development and invagination of crypt structures, proceeds until day 14 in mice [42,43]. Interestingly, targeting of Wnt signaling by addition of exogenous Wnt ligands to fetal cultures stimulates the differentiation into budding structures [40]. This clearly highlights the importance of Paneth cells, which are not present in the embryonic gut, as the main source of WNT in organoid cultures. Similar to these fetal organoids, iPSC-derived organoids are better to study developmental rather than pathophysiological questions due to their maturational status.

3. Organoids as disease models

Intestinal organoids can be used to study the pathophysiology of a variety human diseases including gastrointestinal inflammation (such as inflammatory bowel disease [IBD], celiac disease), infection (*Helicobacter, Salmonella*), malignancy and genetic diseases. Patient-derived organoids further allow drug screening and personalized approaches for treating diseases (Fig. 1).

Intestinal stem cells, which build the fundament of organoids, play an indispensable role for the maintenance of homeostasis and also for the regeneration of damaged epithelium *in vivo*. However, whether the inflammatory environment, created by intestinal inflammation, affects properties of resident intestinal stem cells and thus the phenotype of generated organoids remains uncertain. A recent publication compared the expression profile of organoid cultures either from active lesions or from mucosa under remission from Crohn's disease patients [44]. Interestingly, organoids derived from the different disease activities harbored different expression patterns of stem cell markers and showed alterations in organoid reformation ability. These data indicate that the stem cell phenotype is influenced by the inflammatory environment and that organoids retain the location-specific transcriptional and epigenetic profile of the site of the gut they are derived from. To better understand if and how immune cells affect intestinal stem cell fate or the balance between self-renewal and differentiation, a recent study applied single-cell RNA sequencing to small intestinal organoids. Stimulation of intestinal organoids with key T helper cytokines (such as IFNγ, IL-13, IL-17A, or IL-10) affected stem cell renewal and differentiation in opposing ways: pro-inflammatory signals promoted differentiation, while regulatory cytokines and cells reduced it [45]. Interestingly, this study further identified $MHCII^{+}Lgr5^{+}$ stem cells as non-conventional antigen-presenting cells in co-cultures with $CD4^{+}$ T helper cells.

4. Inflammation

Dysfunction of the intestinal epithelium is believed to result in the excessive translocation of commensal bacteria into the bowel wall that drives chronic mucosal inflammation in Crohn's disease. Studies using epithelial organoids have increased our understanding of the pathophysiology underlying this major form of inflammatory bowel disease. In 2011, the first study demonstrated that caspase-8 deficient intestinal organoids display increased sensitivity towards a proinflammatory kind of cell death (denoted as necroptosis) and additionally an altered Paneth cell homeostasis [46]. Interestingly, Paneth cell defects and increased epithelial cell death are key features of the pathogenesis of Crohn's disease. Accordingly, the authors compared the results obtained in the caspase-8 deficient organoid cultures to data derived from primary human tissue and uncovered a high overlap [46]. This implicates a potential role of caspase-8 dysfunction in the pathogenesis of Crohn´s disease and furthermore that organoid cultures represent a promising novel tool to study human disease mechanisms *in vitro* [46]. Now, 7 years later a recent publication indeed identified germline mutations in the caspase-8 gene in patients with infant-onset inflammatory bowel disease. In line with the previous results [46], patient-derived intestinal organoids had defects in certain cell death processes and displayed increased inflammatory cell death (necroptosis) [46,47] similar to the initial observation in murine organoids. These findings indicate that caspase-8 controls inflammation, innate and adaptive immunity, as well as intestinal barrier integrity in humans. Another study with organoids further shed light on the physiology of Paneth cells as a major factor in the pathogenesis of Crohn´s disease. This study uncovered that stimulation of small intestinal organoids with IFN-γ initially triggered Paneth cell degranulation followed by progressive Paneth cell loss due to cell death [48]. Interestingly, intestinal organoids lacking *Atg16l1*, a risk gene in Crohn's disease, display increased sensitivity towards IFN-triggered inflammatory cell death [49]. These data demonstrate that organoids are useful for understanding the physiology of Paneth cells in particular. This is of significance since Paneth cell dysfunction is a hallmark of ileal inflammation as seen in Crohn's disease, a disease that is still incompletely understood.

5. Cancer

The ability to grow organoids from healthy murine and human intestinal tissue paved the way for organoids derived from patient tumor material. These tumor organoids phenotypically and genetically resemble the tumor epithelium they are derived from and thus represent a promising tool that potentially enables patient-specific drug testing [50]. Tumor-derived organoids grow in a similar man-

ner as the organoids derived from healthy-tissue. Thus, different selection methods, such as the use of special culture media for the growth of tumor cells, have to be applied to allow personalized anticancer treatment. Primary cultures of cancer cells from colorectal cancer (CRC) patients were already established in 2011 and a more recent publication described the generation of the first organoid biobank that consists of primary tumor and matching healthy organoids from CRC patients [51]. Researchers aim to uncover novel drugs, that specifically target tumor cells while leaving normal tissue unaffected. Yet, in order to achieve a statistically significant power to correlate genetics with drug sensitivity, the number of biobanks still needs to be increased. However, it has to be mentioned that just recently Vlachogiannis and colleagues, for the first time, described that drug responses in patient derived-tumor organoids recapitulate the clinical response of the patient [52]. In this study the authors generated a biobank of tumor-organoids derived metastatic colorectal and gastroesophageal tumors from cancer patients that had undergone extensive pretreatment. These organoids were treated with a library containing drugs that are already used clinically or are currently evaluated in clinical trials. A negative predictive value and a positive predictive value of 88% could be observed for the tested substances, again highlighting the role of organoids in the future of medical research and personalized medicine. To validate drug responses in a more complex environment and to also develop new therapeutic options for targeting metastatic tumor growth, a model of xenotransplantation of tumor organoids into immunodeficient mice is currently the state of the art approach. Previous studies have demonstrated that tumor organoids preserve their histological features not only *in vitro* but also after transplantation [53]. The importance of taking advantage of transplanting tissue into its orthotopic environment, in addition to organoid cultures, was recently illustrated in a publication. De Sauvage and colleagues transplanted small intestinal organoids, carrying different combinations of CRC mutations, into the colonic epithelium of mice. Of note, mutations in the tumor suppressor genes *Apc*, *Kras*, *Trp*53 and *Smad*4 were all required to promote progressive tumor growth and metastasis formation [54]. Importantly, only transplantation into their relevant physiological niche in the gut, yield successful dissemination, while subcutaneous injection did not result in the development of metastases. Two other independent studies further confirmed importance of the use of organoids for understanding the adenoma-adenocarcinoma-metastasis cascade *in vivo* [55,56].

6. Organoid cultures from the hepatobiliary system (slow turnover organs)

The liver epithelium comprises two main cell types. Metabolically active hepatocytes and cholangiocytes, which present a barrier to bile, modify its composition and provide functional feedback to hepatocytes. These cells are supported by a constellation of non-epithelial cell types including Kupffer cells, Stellate cells, and sinusoidal endothelial cells forming a complex microenvironment.

The development of hepatobiliary organoids predates the intestinal track. Organoids were first introduced in 2007 to demonstrate the capacity of bipotent hepatoblasts to commit to a biliary lineage [57,58]. These platforms were based on spontaneous differentiation and resulted in organoids with limited function and maturity; however, they provided proof-of-principle for the generation of hepatobiliary organoids. The subsequent development of intestinal organoids (2009) [35] identified almost universal factors, such as R-Spondin 1, promoting long-term culture of epithelial cells in organoid format. Since then, the organoid technology has been extensively used to propagate both hepatocytes and cholangiocytes *in vitro*; originating from adult or foetal primary tissue, or stem cells; and cultured in isolation or combined with multiple non-epithelial cell types. This section will provide an overview of the various available platforms and outline their advantages and disadvantages and illustrate their translational applications.

7. Hepatocytes (hepatic epithelial cells)

In vitro production of bona-fide hepatocytes could provide a unique platform for disease modeling and high-throughput drug screening [59,60]. However, the culture of primary hepatocytes has been limited by loss of the cells' functional properties and proliferation capacity *in vitro* [61]. Organoid systems exhibit a unique capacity to propagate primary epithelia, which could address these challenges, provided the resulting cells maintain high functionality. To meet this requirement various approaches have been adopted, all of which take advantage of the physiological mechanisms of liver development and regeneration, to generate functional hepatic organoids.

The first generation of liver-derived organoids originated from adult liver stem cells, located in the bile ducts of organs with end-stage liver disease [5]. This platform combined growth factors required for organoid propagation (Wnt, BMP/TGF-β, EGF), which were adapted from the intestinal organoid culture conditions, with key pathways for liver development and homeostasis (HGF, FGF10, Notch) [62]. The resulting organoids retained their adult stem cell phenotype, but could differentiate towards a hepatic fate, upon withdrawal of stimuli required for biliary lineage commitment (Wnt, Notch) [63], both in human and mouse. This system provided a unique platform for studying the role of adult stem cells in liver disease and repair from injury. However; since the events controlling the transition of adult stem cells to hepatocytes are not fully described, differentiated hepatic organoids maintained some differences in terms of function and marker expression compared to primary cells.

To address challenges related to differentiation efficiency, the second generation of hepatic organoids was based on direct propagation of primary hepatocytes [64,65]. This was achieved by combining lessons from the hepatic differentiation of adult stem cell organoids with pathways controlling liver regeneration after injury, such as FGF7 [66,67] or TNFα [68]. These conditions resulted in successful propagation of primary hepatocytes from foetal or adult whole liver, in both human and mouse. The resulting hepatocyte organoids expressed markers and function at levels comparable to primary tissue, providing a promising platform for translational applications, such as drug screening.

Despite their advantages, the use of primary organoids is restricted by access to tissue, especially in rare disorders, where non-invasive diagnostic modalities are not available. iPS cells can address this limitation since they can be derived from a wide variety of sources, with minimally invasive procedures (e.g. blood) [69]. In addition, 3D conditions promote maturation of iPS-derived hepatocytes [70] rendering organoids an advantageous system compared to conventional monolayer culture. Consequently, various protocols for the differentiation of iPS cells into hepatocyte organoids were designed, which recapitulate key stages of liver morphogenesis *in vitro* by providing developmentally relevant cues. Physiologically, bipotent hepatic progenitors, known as hepatoblasts, arise from the anterior foregut endoderm; and commit to a hepatic or biliary lineage based on stimuli as TGF-β or Notch. To reproduce these events 2 approaches have been adopted. In most platforms, endoderm induction and hepatic specification are performed in monolayer and the resulting hepatoblasts are transferred in protein-rich matrices to form 3D aggregates and mature. These systems generate relatively homogeneous populations of hepatocyte organoids [71–74] compatible with mechanistic studies and large-scale analyses. Alternative methods complete the whole differentiation in 3D, starting from iPS spheroids and

resulting in heterogeneous organoids comprising hepatocytes and cholangiocytes or mesenchymal cells [75–77]. These heterogenous systems are more physiological, recapitulating cell-to-cell interactions which play an important role for hepatocyte maturation *in vivo*. However, the factors promoting maturation are not fully defined rendering mechanistic studies more challenging.

In summary, iPS-derived organoids recapitulate liver morphogenesis and address issues related to tissue access; providing valid platforms for mechanistic studies on development and rare disorders. However, they remain foetal and do not acquire a fully mature phenotype compared to their primary counterparts.

8. Multicellular organoids

The crosstalk between hepatocytes and multiple other liver cell types plays a key role for liver development [78], pathophysiology [79–81] and disease [82,83]. These dynamic interactions can be captured by advanced multicellular organoid systems, based on co-culture of hepatocytes with other cell types. The simplest iteration of this approach involves two-cell-type systems, which have been successfully used for mechanistic studies in liver fibrosis [84], xenobiotics metabolism [85] and hepatocyte-to-stroma crosstalk [86]. To explore more complex interactions, platforms involving iPS-derived hepatoblasts, endothelial and mesenchymal cells have been developed, which take advantage of the intrinsic ability of liver cells to self-assemble into complex 3D structures resembling the foetal liver-buds [87]. This interplay between various cell types and the surrounding matrix provides a more physiological representation of the *in vivo* micro-environment compared to previous platforms, rendering these multicellular systems optimal for providing a more holistic overview of liver development and disease. Indeed, the potential of multicellular organoid culture for studying processes as drug-induced liver injury [88], viral hepatitis [89,90] and liver development [87] has already been demonstrated; however, these platforms remain technically more challenging in terms of downstream analyses and mechanistic studies compared to their monoculture counterparts.

9. Cholangiocytes (biliary epithelial cells)

The main task of cholangiocytes is maintaining the luminal homeostasis of the bile ducts. 3D structures encompassing a lumen are essential to preserve these functions *in vitro*. This requirement explains the limitations of conventional cholangiocyte monolayer cultures in terms of cell expansion and function, which resulted in a lack of appropriate *in vitro* platforms which restricted the development of new therapies for cholangiopathies. The potential of cholangiocyte organoids to address this pressing clinical need by providing adequate *in vitro* models for biliary disorders made them the first type of organoids to be developed in the field of hepatology.

Like hepatocytes, cholangiocyte organoids can be generated from primary or iPS cells. Primary systems are based on two complementary approaches. Conditions based on canonical Wnt signaling are used to propagate adult liver stem cells with a biliary phenotype [5]. These cells express basic cholangiocyte markers and are well suited for studies on the transition between hepatic and biliary lineage; however, they do not fully recapitulate the function of mature *in vivo* cholangiocytes. Conversely, systems based on non-canonical Wnt signaling promote the expansion of mature cholangiocytes with enhanced function but limited differentiation capacity to other lineages [91], making these cells highly suitable for regenerative medicine application [92]. Consequently, Wnt seems to be a master regulator determining a mature vs. stem-cell phenotype.

iPS-based platforms can be monocellular [93] or multicellular [94]. Both systems are based on developmental cues like TGF-β, Notch, Wnt, and FGF to mimic biliary development and generate cholangiocytes. In multicellular systems biliary fate commitment of hepatoblasts is further enhanced through the interaction with OP9 cells, recapitulating the crosstalk with the periportal mesenchyme [95]. Multicellular cultures are more physiological however the paracrine signals between cells remain unknown. On the contrary, monocellular systems are chemically defined [24], reducing culture variability and enabling mechanistic studies. In keeping with hepatocyte organoids, iPS-derived cholangiocyte organoids address problems of access to tissue and are suited for developmental studies or modelling rare disorders. However, the cells retain foetal characteristics and display reduced functionality compared to primary cells.

10. Applications

10.1. Disease modeling and drug screening

Hepatobiliary organoids have been used to model several monogenic liver diseases including cystic fibrosis [93,94], polycystic liver disease [93], Alagille syndrome [75], Wilson disease [96] and α1-antitrypsin deficiency [5]. These patient-derived organoids recapitulate the disease phenotype *in vitro* and can be used as drug screening platforms for the development of novel therapeutics towards personalized medicine. The repurposing of the cystic fibrosis transmembrane conductance regulator gene (CFTR) correctors VX809 (lumacaftor) and VX770 (ivacaftor) as a potential therapy for cystic fibrosis liver disease, after screening on cholangiocyte organoids derived from patients affected by cystic fibrosis [93], provide an example of this approach; while, paracetamol-mediated fibrosis has been studied using liver organoids comprising HepaRG and stellate cells [85].

Both hepatocellular carcinoma and cholangiocarcinoma have been associated with expansion of adult stem cells in the liver. Based on this observation, the platform for propagating adult liver stem cells was adapted to expand primary liver tumor organoids *in vitro* (tumoroids) [97], from both cholangiocarcinoma and hepatocellular carcinoma. These cells recapitulate the mutational burden and genomic landscape of the tumor of origin in culture. These properties render the tumoroid platform highly suitable for drug sensitivity testing in a patient-specific fashion and provide a unique tool for personalized medicine in cancer. The identification of the ERK inhibitor SCH772984 as a novel chemotherapeutic agent provides proof-of-principle for the potential of this system.

Finally, organoid systems can be used for studying multifactorial, acquired and infective disorders. Indeed, a Biliary Atresia model has been developed by exposing mouse cholangiocyte organoids to the plant toxin biliatresone, which has been associated with the disease, and demonstrating disruption of their 3D architecture [98]. In addition, hepatic organoids have been used to model hepatitis B [89] and C [90] infection. These organoids recapitulate virus-to-host interactions more faithfully compared to monolayer systems due to superior cell polarization in 3D conditions. The potential of hepatobiliary organoids to originate from primary tissue overcomes maturity issues and could potentially preserve the effect of environmental or epigenetic factors in culture. This renders primary organoids optimal for studying complex multifactorial disorders.

10.2. Regenerative medicine

The treatment of hepatobiliary disorders is restricted by the shortage of healthy tissue suitable for transplantation. Organoids represent a source of functionally mature, easily expandable cells that could address this issue. Indeed, transplantation of iPS-derived liver-buds rescued lethal liver failure in animal models [87], providing the first proof-of-principle for the feasibility and efficacy

of highly-organized, multicellular organoids. Engraftment of primary hepatocyte organoids has also been demonstrated in mice [5,64,65], addressing some of the safety concerns associated with the use of iPS cells [99]; however, these cells can be derived only from whole-liver perfusion, rendering cell-based therapy using autologous cells challenging. Furthermore, primary human cholangiocyte organoids have been combined with biodegradable scaffolds to generate bioengineered bile ducts, capable of replacing the native bile duct of immunocompromised mice [91]. These cells can be derived from multiple sites (gallbladder tissue, endoscopic retrograde cholangiopancreatography [ERCP] brushings) and small quantities of starting material overcoming issues relating access to tissue for the generation of autologous organoids. Finally, advances in gene editing have enabled correction of genetic defects using the CRISPR/Cas9 [100] technology in patient organoids. Although still at an early stage, considered collectively, these advances hold promise for regenerative medicine and the clinical translation of autologous cell-based therapy using patient specific organoids [101].

10.3. Mechanistic studies

The use of organoids holds great promise not only for translational research but also for mechanistic studies on tissue biology. Organoid cultures recapitulate tissue architecture and complex cell-to-cell and cell-to-matrix interactions more efficiently than monolayer culture; while they remain a more controlled, human system, compatible with large-scale analysis under well-defined conditions compared to *in vivo* experiments. These attributes provide significant advantages for addressing developmental and biological questions that have proven challenging to tackle using conventional methods. A characteristic example illustrating this potential is the use of multicellular organoids to model liver organogenesis in 3 dimensions, using in iPSC-derived liver-buds [102], which has not been possible with previous conventional systems. This model was subsequently used for transcriptomic analyses at single-cell resolution, which allowed to dissect the interlineage communication controlling human liver organogenesis and vascularization; while similar approaches, are starting to be used to explore multiple other aspects of liver morphogenesis, remodeling and pathophysiology [103].

11. Challenges and future directions

Recent technical advances in culturing human organoids will further promote their use to understand the pathophysiology of gastrointestinal or hepatic diseases. Genetic editing based on CRISPR/Cas9 technology has been successfully established in the organoid cultures derived from colorectal cancer patients [104,105]. In addition to the CRISPR/Cas9 system, gymnosis, a process of introducing modified or locked nucleic acids complementary to a specific gene or miRNA, in order to knock down gene expression, has also been applied to organoid cultures [106]. To store human or modified organoid cultures in a biobank, companies have developed specialized freezing media for cryopreservation. Several studies already visualized a high genetic overlap between cryopreserved organoid cultures and corresponding biopsy specimen of CRC patients [107].

Despite the advances in the organoid field in the last decades, several challenges remain to be tackled. A major limitation for mechanistic studies and regenerative medicine applications is the use of poorly defined matrices, consisting of extracellular matrix proteins (Matrigel). This approach has some disadvantages such as that Matrigel is undefined (the concentrations of growth factors and other biologically active components in differs from batch to batch). The development of chemically defined hydrogels and high-throughput systems, which enable to up-scale organoid production in a setting compatible with Good Manufacturing Practice (GMP), represent significant steps towards addressing such challenges. Interestingly, the chemical and physical properties of some of these new hydrogels can be spatially and temporally modulated to recapitulate ECM properties in health and disease (e.g. fibrosis) [103,108], adding a new level of control over organoid cultures [109]. A recently published study described a method to cultivate organoids in a hanging drop without embedding it in Matrigel [110]. This might further provide an attractive alternative to currently used protocols. Another key advantage of organoids is the generation of complex multicellular structures recapitulating the gut/liver microanatomy; however, the presence of multiple cell types renders downstream analysis and mechanistic studies technically challenging. The use of high-resolution techniques, such as single cell RNA sequencing (scRNAseq) overcomes these challenges. While cultivating organoids in a 3-dimensional orientation is important to recapitulate *in vivo* morphology and function of the originated organ, the 3D structure of organoids hinders an approach of targeted application of microbes or cytokines to the apical (luminal) surface of the organoid epithelium. One possibility to bypass this problem is the achievement of 2D monolayer cultures of organoids, still containing all different epithelial cell types [111–113]. This method is especially convenient to study host-microbe interaction and therefore the pathogenesis of gastrointestinal infections. Moreover, these monolayers of former 3D organoids can be treated to enrich epithelial cells of the secretory lineage to further explore how soluble factors released by these cells modulate the adhesion of microbes [114,115]. Another possibility to circumvent problems regarding organoid orientation is to perform microinjections of microbes or other factors into the lumen of organoids (which represents the intestinal/biliary lumen *in vivo*). This will help to better understand how these factors affect physiological functions of epithelial cells and to shed light into the pathophysiology of infectious diseases [11,116,117]. Pathogens including viruses and bacteria are able to persist in a viable state in the inner lumen of the organoids. Microinjection of pathogens into the organoid lumen is also used to analyze the infection of the stomach with *Helicobacter pylori* [118,119].

Finally, improving the maturity and microarchitecture of organoids has been an area of ongoing research. Indeed, strategies to improve maturity have been investigated, ranging from organoid vascularization, to application of mechanical stimuli and development of culture setting endorsing a physiological spatial relationship between cells types. 3D printing, microfluidic devices and organ-on-chip platforms allow the creation of complex microenvironments with mechanical properties and growth factor gradients resembling the native niche. Furthermore, gut and liver organoids could be used to generate two-organs-on-chip devices [120] and model the enterohepatic axis [121]. This advances the use of organoids to more holistic models taking into account the complex interplay between multiple organs.

12. Conclusion

In conclusion, although gastrointestinal and hepatobiliary organoids have been developed only in the last decade, they have already proven to be an invaluable tool for drug screening, disease modelling and regenerative medicine. Challenges still remain; however, recent advances in bioengineering, organ-on-chip technology, single-cell analyses and synthetic matrix development hold great promise for addressing current limitations and advancing the field even further, both in terms of mechanistic studies and clinical translation.

Conflict of interest

MFN has served as an advisor for Pentax, Giuliani, PPM, MSD, Takeda and Boehringer. All other authors have nothing to disclose.

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