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Review article

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Advances in liver and pancreas organoids: how far we have come and where we go next

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Abstract

Over the past decade, advances in organoid culturing methods have enabled the growth of three-dimensional cellular cultures in vitro with increasing fidelity with respect to the cellular composition, architecture and function of in vivo organs. The increased accessibility and ability to manipulate organoids as an in vitro system have led to a shift in the landscape of experimental biology. Whether derived from stem cells or tissue-resident cells, organoids are now routinely used in studies of development, homeostasis, regeneration and disease modelling, including viral infection and cancer. These applications of organoids are highly relevant for gastrointestinal tissues, including the liver and pancreas. In this Review, we explore the current and emerging advances in liver and pancreas organoid technologies for both discovery and clinical translation research and provide an outlook on the challenges ahead.

Sections

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Key points

- Modelling the liver and pancreas using organoid technology provides an accessible, often human-based system for research into fundamental questions regarding their embryonic development, function, disease modelling and clinical applications.
- Liver and pancreas organoids are generated utilizing multiple sources as starting material, including pluripotent stem cells, embryonic, fetal and adult stem cells, and adult differentiated cells, each with specific advantages and disadvantages.
- Current liver and pancreas organoid models have enabled a greater understanding of both acquired and inborn diseases that are not possible with in vivo models.
- Advances in co-culture technologies are leading to the generation of organoids with multiple interacting cell populations found within the in vivo liver or pancreas enabling the production of higher fidelity models with more mature cell types.
- Standardization of protocols, improvement of organoid architecture accuracy and transition towards chemically defined extracellular matrices will drive advances in the liver and pancreas organoid field.

Introduction

Research using organoids has increased considerably since the first self-organizing, three-dimensional systems were described for cortical tissue[1] and small intestine[2] in 2008 and 2009, respectively. In the following years, organoid protocols to model a multitude of different tissues have been described and, in many cases, adapted for human cells[3]. In this Review, we differentiate between organoids and other three-dimensional culture techniques, such as spheroid cultures and slice cultures. In contrast to spheroid cultures, organoids make use of an extracellular matrix (ECM) to mimic physiological mechanical cues and facilitate cell polarization[4]. Slice cultures are not expandable and do not require self-organization, a key aspect of organoid establishment. The culture conditions for organoids are specific for their organ of origin; therefore, here we define organoids in general terms as in vitro three-dimensional multicellular cultures derived from tissue-resident stem or progenitor or differentiated cells, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) capable of self-renewal and self-organization that recapitulate the functionality of the tissue of origin. The definitions and nomenclature describing hepatic, biliary and pancreas organoids are extensively described elsewhere[4].

The liver and pancreas exert a multitude of functions to regulate whole-body metabolic homeostasis. Two major epithelial cell types populate the tissue in the liver: hepatocytes and ductal cells (or cholangiocytes). Hepatocytes and ductal cells display distinct patterns of apicobasal polarity and are physically and functionally connected. Bile duct cells align their apical domains towards a shared lumen, forming bile ducts. Unlike the uniform apicobasal polarity of bile duct cells, a single hepatocyte features multiple basal and apical domains, generating a network of narrow tubular lumina called bile canaliculi. Functionally, hepatocytes are involved in xenobiotic metabolism, detoxification, bile synthesis and glycogen storage, whereas ductal cells modify and regulate the transport of bile[5–7].

The adult pancreas is functionally divided into exocrine and endocrine components. The exocrine pancreas produces digestive enzymes (amylase, lipase and proteases) and consists of acinar cells that secrete these enzymes and ductal cells that transport them to the digestive tract[8–12]. The endocrine function is carried out by the islets of Langerhans, which are clusters of hormone-producing cells scattered throughout the pancreas, responsible for glucose homeostasis. β-Cells, in particular, are responsible for secreting insulin, which lowers blood glucose levels, whereas α-cells produce glucagon, which raises glucose levels in the blood[13,14]. Together, the liver and pancreas function as accessory digestive organs (food does not pass through them). Bile (synthesized by hepatocytes in the liver) and digestive enzymes (produced by the acinar cells of the pancreas) are collected by a branching network of bile and pancreatic ducts and transported to the gallbladder and duodenum, respectively, to aid in digestion.

The aforementioned cells function in conjunction with closely associated endothelial, immune and mesenchymal cells to regulate carbohydrate, lipid and xenobiotic metabolism[15–27]. The ability of the liver and pancreas to function relies on the correct development and maintenance of the different cell types (Box 1). Perturbation of the developmental or homeostatic programmes of these cells can cause a variety of liver and pancreas diseases in which the function of the whole organ can be diminished.

The most common liver diseases include primary liver cancers and metabolic dysfunction-associated steatotic liver disease (MASLD) (previously known as non-alcoholic fatty liver disease), the prevalence of which is rapidly increasing globally[28]. Cholestatic liver disease and liver fibrosis are also among the most common liver disorders affecting individuals worldwide[29]. Common pancreatic diseases include diabetes mellitus, pancreatic ductal adenocarcinoma (PDAC), pancreatitis and cystic fibrosis[30–32]. Most of the knowledge related to the development and stages of the mentioned diseases can be attributed to patient tissue biopsy samples and mouse models. Even though very informative, analyses of fixed tissue sections provide a snapshot of the disease stage, whereas the fourth dimension, time, is missing. As a model system, organoids can be capable of long-term culture. They recapitulate key properties such as three-dimensional tissue architecture, gene expression profiles and function, and are amenable to live cell imaging and genetic manipulations; therefore, they can provide the missing time dimension. For this reason, the use of organoids for research into basic regulatory mechanisms of development, homeostatic processes and perturbations during disease progression is proving to be a powerful tool. However, different cell populations have proven to have different levels of success when cultured as organoids.

Here, we review the advances in liver and pancreas organoid models and discuss to what extent different liver or pancreas cell types can be cultured in vitro, the functional maturity of these cells and what insights can be gained from these models.

Modelling liver and pancreas using organoid technology

Organoid systems have been described for many tissues of the body; currently, not all of these tissues can be modelled from both pluripotent stem cells (PSCs) and tissue-resident cells. For example, protocols for the brain and optic cup require PSCs for derivation. Conversely, organoids from tissues such as the mammary gland, endometrium and oesophagus are initiated from tissue-resident cells. The liver and pancreas are among the subset of organs for which organoid systems have

been derived from PSCs and tissue-resident cells, including embryonic and adult stem or differentiated cells[33].

Formation of epithelial liver and pancreas organoids has been achieved by several groups (Table 1). The proliferation, differentiation and spontaneous self-organization observed during organoid formation rely on supplementing the culture medium with specific exogenous growth factors. During organoid derivation from PSCs (ESCs or iPSCs), cells are cultured with growth factors that mimic the dynamic temporal signals that cells are exposed to during embryonic patterning from the inner cell mass to endodermal progenitor to committed liver or pancreas cell. These cells are pre-differentiated in 2D culture and then fully differentiated in 3D culture. In the case of organoids derived from tissue-resident cells and/or progenitors, the progenitors are enriched following enzymatic digestion of primary tissue and then cells are either embedded in or overlayed on ECM, or placed in ECM and cultured using an air–liquid interface approach, amongst other methods (for details of different methodologies we refer the reader to extended reviews on this topic[34–37]). In all cases, the culture medium is designed to provide the stem cell niche signals that are present during homeostatic self-renewal or tissue damage repair. These progenitors can be isolated from embryonic or adult tissues and diseased states. Differences in the starting material (cell or tissue source) and culture conditions (stimulation of specific pathways using growth factors and inhibitors) lead to the generation of organoids with different morphologies and cell compositions (Figs. 1 and 2).

Box 1 | Cell types in the liver and pancreas, including parenchymal and non-parenchymal cells

Liver cell types
- Epithelial cells (hepatocytes and ductal cells)[5–7]
- Hepatic stellate cell[15,16]
- Sinusoidal endothelial cell[17–19]
- Portal fibroblast[20,21]
- Kupffer cell[18,22,23]

Pancreas cell types
- Acinar cell[8–10]
- Ductal cell[11,12]
- Islets (α, β, δ, ε and pancreatic polypeptide cells)[13,14]
- Pancreatic stellate cell[24,25]
- Endothelial cell[26,27]

ESC and iPSC

The primary tissue used during the isolation of tissue-resident progenitors for human-based organoids is a limited resource. Procedures to obtain primary material from liver and pancreas biopsy samples or fetuses carry a high risk of complications, and, therefore, the samples for organoid derivation predominantly come from organ donors, patients with an underlying disease or aborted pregnancies. The use of PSCs to generate organoids overcomes this limitation, as cell numbers can be expanded vastly before differentiation. Liver and pancreas PSC-derived organoids are generated by differentiation through a stepwise process in which they become increasingly cell-fate restricted, first to EPCAM$^+$ definitive endoderm and then HNF6$^+$ primitive foregut before being driven towards a liver or pancreas progenitor population, and then placed into three-dimensional culture to form liver or pancreas organoids[3].

Liver. In 2013, Takebe and colleagues demonstrated that hepatic lineage-specified human iPSCs could self-organize into embryonic liver bud tissue in the presence of bone marrow mesenchymal stem cells and human umbilical vein endothelial cells[38]. This tissue was engrafted into mice with severe combined immunodeficiency (SCID), upon which it proliferated and developed structural and functional characteristics of the adult liver, with the development of hepatic cord-like structures expressing the mature hepatocyte markers albumin, cytokeratin 8 and cytokeratin 18, along with a decrease in the embryonic marker α-fetoprotein. These findings demonstrated that iPSCs can be differentiated into functional liver bud cells[38]. Subsequent work in vitro revealed a transitional state in which definite endoderm markers such as CXCR4 were downregulated, but the hepatic progenitor marker HNF4α was not yet upregulated. Utilizing cells from this transitional state, along with the generation of iPSC-derived stromal cells (equivalent to bone marrow mesenchymal stem cells) and iPSC-derived endothelial cells, enabled the generation of vascularized liver bud organoids in which all three required cell populations were derived from feeder-free human iPSCs[39]. Further to this, methods were adapted to use solely chemically defined and animal origin-free media, opening the way for potential clinical applications for liver bud organoids[40].

In addition to forming liver buds reminiscent of embryonic stages, liver organoids of a more mature stage have also been derived from iPSCs. Ductal liver organoids have been obtained from human iPSCs via two strategies: one used a stromal cell line to inhibit NOTCH signalling and drive differentiation from bipotent hepatoblasts to ductal organoids[41], and the other first differentiated cells to ductal progenitors through the addition FGF10, retinoic acid and activin A to the culture medium followed by a final differentiation to a more committed ductal lineage with epidermal growth factor (EGF)[42]. Primary human hepatocytes have often proven difficult to culture in vitro[43], but progress has been reported using PSCs to generate hepatocyte organoids. In 2019, human ESC-derived bipotent liver organoids were developed that, following engraftment into SCID mice, formed cell aggregates expressing hepatocyte markers, including HNF4α and albumin[44]. These bipotent liver organoids could also be differentiated in vitro into ductal lineage organoids expressing mature cholangiocyte markers, including keratin 19, or hepatocyte lineages, although the levels of functional hepatocyte activities, including albumin secretion, urea production and CYP3A4 metabolic activity, were markedly lower than primary human hepatocytes[44]. In 2025, Saiki and colleagues reported the generation of iPSC-derived hepatocyte organoids containing liver sinusoidal endothelial-like cells, which, when transplanted, enabled the rescue of coagulation factor defects in mice[45]. These advances to form organoids that express markers of bipotent liver cells, ductal lineage and hepatocyte lineage cells from PSCs using chemically defined methodologies laid the foundations for clinical applications and toxicology studies (applications have been extensively reviewed elsewhere[36]). However, currently, these models do not recapitulate the functional maturity of their in vivo counterparts, and this is a key challenge to be addressed.

Pancreas. During development, pancreatic progenitors give rise to all five endocrine lineages of the islets of Langerhans (α, β, δ, ε and pancreatic polypeptide) as well as the exocrine acinar and ductal cells[13]. In vitro, PSCs are directed to definitive endoderm, which is then

Table 1 | Comparative list of developments in liver and pancreas organoid models including their source and composition

Tissue modelled	Starting material	Organoid composition	Species	Derived from	Ref.
Intrahepatic cells (liver)	Tissue-resident cells	Ductal cells; can be transdifferentiated to hepatocytes	Mouse	Damaged adult LGR5$^+$ cells; adult healthy ducts	Huch et al. (2013)[60]
		Ductal cells; can be transdifferentiated to hepatocytes	Dog	Adult healthy fine-needle biopsy sample	Nantasanti et al. (2015)[80]
		Ductal cells; can be transdifferentiated to hepatocytes	Human	Adult healthy ducts	Huch et al. (2015)[62]
		Hepatocytes	Mouse	Adult healthy hepatocytes	Hu et al. (2018)[70] Peng et al. (2018)[83] Dowbaj et al. (2025)[84]
		Hepatocytes	Human	Adult healthy hepatocytes	Igarashi et al. (2025)[85] Yuan et al. (2025)[86]
		Hepatocytes	Human	Fetal healthy hepatocytes	Hu et al. (2018)[70]
		Ductal cells Hepatocytes	Mouse	Embryonic LGR5$^+$ hepatoblasts Embryonic bulk hepatoblasts	Prior et al. (2019)[72]
		Hepatoblasts; can differentiate to ductal or hepatocyte lineage	Human	Fetal liver tissue	Wesley et al. (2022)[68]
		Ductal cells; can form branched structures	Human	Adult healthy ducts	Roos et al. (2022)[82]
	PSCs	Ductal cells	Human	iPSCs	Ogawa et al. (2015)[41] Sampaziotis et al. (2015)[42]
		Liver bud	Human	PSC endoderm cells, PSC endothelial cells; HUVECs, MSCs	Takebe et al. (2013)[38] Takebe et al. (2017)[39]
		Ductal cells Hepatocytes	Human	ESCs	Wang et al. (2019)[44]
		Multizonal hepatocytes	Human	iPSCs	Al Reza et al. (2025)[159]
Pancreatic cells	Tissue-resident cells	Ductal cells	Mouse	Adult healthy ducts	Huch et al. (2013)[61]
		Embryonic progenitors	Mouse	Embryonic pancreas	Greggio et al. (2013)[74] Sugiyama et al. (2013)[73]
		Embryonic progenitors	Human	Fetal pancreas	Bonfanti et al. (2015)[75]
		Ductal cells	Human	Adult healthy ducts	Boj et al. (2015)[98]
		Ductal cells	Human	Adult healthy ducts	Loomans et al. (2018)[99]
		Ductal cells	Human	Adult healthy ducts	Georgakopoulos et al. (2020)[63]
		Islets	Mouse	PROCR$^+$ progenitors or primary endothelial cells	Wang et al. (2020)[101]
	PSCs	β-Cells	Human	iPSCs ESCs	Pagliuca et al. (2014)[46]
		Exocrine pancreas	Human	ESCs	Huang et al. (2015)[50]
		Acinar cells Ductal cells	Human	ESCs	Huang et al. (2021)[53]
		Islet-like organoids producing insulin	Human	Gastric stem cells from human stomach	Huang et al. (2023)[147]
Multitissue organoid	Tissue-resident cells	Hepatocytes Ductal cells Portal fibroblasts	Mouse	Adult healthy hepatocytes, ductal cells and portal fibroblasts	Dowbaj et al. (2025)[84]
		Hepatocytes Ductal cells Portal fibroblasts	Human	Patient-derived healthy hepatocytes, ductal cells and portal fibroblasts	Huch et al. (2025)[86]
		Hepatocytes Ductal cells		Patient-derived healthy hepatocytes	Marsee et al. (2021)[170]

Table 1 (continued) | Comparative list of developments in liver and pancreas organoid models including their source and composition

Tissue modelled	Starting material	Organoid composition	Species	Derived from	Ref.
Multitissue organoid (continued)		Hepatocytes Fibroblasts		Patient-derived healthy hepatocytes, and fibroblasts	Mallanna et al. (2024)[160]
	PSCs	Liver bud Pancreas Common bile duct	Human	Anterior gut PSCs Posterior gut PSCs	Koike et al. (2019)[167]

ESC, embryonic stem cell; HUVEC, human umbilical vein endothelial cell; iPSC, induced PSC; LGR5, leucine-rich repeat-containing receptor 5; MSC, mesenchymal stem cell; PROCR, protein C receptor; PSC, pluripotent stem cell.

differentiated to NKX6.1$^+$ and PDX1$^+$ pancreatic progenitors before differentiation into endocrine or exocrine lineages and then onto specific pancreatic cell types[46]. A major focus of driving PSCs to a pancreatic fate is the successful generation of insulin-producing β-cells. During the period 2014–2015, multiple groups demonstrated high-efficiency reprogramming of human iPSCs to insulin-expressing β-cells, which performed glucose-stimulated insulin secretion, confirming that endocrine pancreatic cells could be generated from PSCs with functional characteristics[46–48]. The replating of pancreatic progenitors derived from iPSCs into a three-dimensional culture generated expandable pancreatic progenitor organoids. These organoids, when compared with the two-dimensional cells, transcriptionally resemble human fetal pancreas tissue better, providing a potential system to investigate early fetal pancreatic development[49].

Generation of exocrine pancreas organoids from PSCs was first demonstrated through human ESC-derived pancreatic progenitor organoids, which could be expanded as pancreatic progenitors or further differentiated into a mixed ductal (CA2$^+$) and acinar (CPA1$^+$) organoid culture[50]. In 2021, several groups reported the generation of pancreatic duct-like organoids (PDLOs) from human induced PSCs (hiPSCs) following a stepwise protocol in which PSCs were differentiated into pancreatic progenitors in two-dimensional culture before final differentiation into PDLOs through NOTCH pathway activation[51–53]. Furthermore, acinar organoid cultures could be obtained from these progenitors by activating the canonical WNT pathway with simultaneous inhibition of NOTCH and Hedgehog pathways[53]. In a 2024 study, branched hiPSC-derived pancreas organoids were developed by combining 10 days of two-dimensional culture, followed by 4 days in microwell plates to promote cell aggregation and then 5 days of suspension culture[54]. These branched structures consist of spatially distinct progenitor cells, trunk cells and acinar cells, which recapitulate the in vivo architecture better than previously reported exocrine organoids that present as epithelial spheres.

The development of ductal and acinar cell organoids also enabled an investigation into drivers of PDAC in which pancreatic progenitor cells expressing the PDAC oncogene *GNAS*^{R201C} increased ductal organoid size and were expandable as stable cultures over 15 passages, which was not observed in the acinar organoids, demonstrating lineage-specific effects[55]. With the generation of multiple pancreatic cell types within organoids, the further use of PSC methods to generate co-cultures with increased complexity might enable the generation of mature β-cells whose capacity to generate insulin and respond to glucose is comparable to the capacity of their in vivo counterparts.

A major challenge faced when using PSC-derived organoids is one of maturity. PSC methods use cells that are sourced from pluripotent embryonic cells before germ layer formation or reprogrammed from somatic cells to induce pluripotency. This immaturity makes the stepwise methods to generate PSC organoids possible but results in the final differentiated organoids retaining immature characteristics, such as the expression of markers of other endodermal tissues (for example, the expression of colon markers, including CDX2 and CDH17) in liver hepatocyte organoids[56]. Despite the additional time and steps required for generating PSC-derived organoids, the ability to generate iPSC-derived organoids from an individual's skin cells without taking invasive biopsy samples enables the creation of personalized organoid–patient avatars, including those from patients with rare conditions, which could greatly improve translational research. An important benefit is the potential to co-create endothelial and stromal compartments within a culture system, which enables investigations into how interactions between different cells influence cellular differentiation programmes.

Tissue-resident cells challenge the Hayflick limit

In contrast to PSC-derived organoids that must be guided through lineage fate restrictions from pluripotency to the organ of choice, fate-committed tissue-resident stem, progenitor or differentiated cells can be isolated from the adult or embryonic organ to generate liver or pancreas organoids. In 1961, the Hayflick limit proposed that adult or embryonic tissues cannot expand indefinitely unless genetically transformed[57,58]. In 2013 and 2015, 50 years later, the establishment of adult-tissue-derived organoids from mouse and human liver and pancreas challenged that limit, as it was proven that both liver and pancreas human tissue grown as organoids would expand long-term under serial passaging while retaining their genetic stability over time[59–63]. These organoids are not only genetically stable but can also largely maintain the epigenetic profile of the in vivo cells[64,65] and show a reduced tumorigenic potential compared with PSC-derived organoids[66].

Embryonic tissue-resident progenitors

Organoids derived from embryonic liver and pancreatic tissue-resident progenitor stem cells recapitulate aspects of the in vivo development of the organs under controlled in vitro conditions. These liver and pancreas embryonic tissue-resident progenitors represent cells already primed to differentiate into the cell types of the adult organs as they would during development, without the need for reprogramming using PSC methods.

Embryonic liver. During embryogenesis, the liver bud is initially populated by bipotent progenitors known as hepatoblasts. A key event of liver morphogenesis and organogenesis occurs from embryonic day (E) 10.5 to E13.5 in mice, and 7–8 weeks after conception in humans, when the hepatoblast population begins to differentiate into hepatocytes and cholangiocytes[67,68]. Historically, hepatocyte cells have been

difficult to culture from adult tissue, from which primary isolations often only survive for a short time[43,69]. However, this has been overcome, to an extent, by using embryonic progenitors primed to undergo hepatocyte differentiation. In 2018, Hu and colleagues established culture conditions to generate fetal human hepatocyte organoids from hepatocytes isolated from fetal livers at 11–20 weeks after conception[70] (Fig. 1b). These fetal hepatocyte organoids were capable of long-term expansion and replicated structural aspects of mature hepatocytes, including microvilli and bile canaliculi structures, and functional aspects, including albumin secretion and CYP3A4 activity. After engraftment into mice, they remained proliferative at 90 days and continued to express the hepatocyte markers albumin and CYP2E1 (ref. 70). CRISPR–Cas9 genome engineering has also been used successfully on human fetal hepatocyte organoids, demonstrating that embryonic liver tissue-resident progenitor organoids are amenable to such treatment, and opening the potential for further genomic engineering of these primary cultures[71]. A similar methodology was shown to support the generation of hepatocyte organoids from embryonic mouse livers; here, *Lgr5* was first identified as a marker of bipotent E10.5 hepatoblasts in vivo, which, when isolated, could be driven to form either ductal or hepatocyte organoids in vitro[72] (Fig. 1a). Single-cell RNA sequencing (scRNAseq) of the developing embryonic liver further highlighted the role of LGR5$^+$ stem cells and the importance of WNT pathway activation. Through direct stimulation of the WNT pathway, EPCAM$^+$ human fetal hepatoblasts organoids could be maintained long-term whilst retaining the ability to later differentiate into hepatocyte and ductal lineages[68] (Fig. 1b). Together, these systems provide experimental models in which the plasticity of embryonic hepatoblasts can be investigated.

Embryonic pancreas. The mouse and human embryonic pancreas contains multipotent SOX9$^+$ progenitors, which amplify and differentiate into both the exocrine and endocrine lineages. Within these progenitors, NGN3$^+$ subpopulations arise to form the endocrine lineages of the islets of Langerhans, while NGN3$^-$ cells form the exocrine acinar and ductal cells[13]. In 2013, two studies of embryonic mouse pancreatic progenitor organoids were reported. One study found that SOX9$^+$ NGN3$^-$ progenitor cells isolated at E11.5 could form organoids capable of short-term expansion but required coculture with pancreatic mesenchymal cells[73]. Another study found that E10.5 embryonic mouse pancreas cells could generate pancreatic progenitor organoids along with spontaneously differentiating and morphologically distinct branched organoids, which could be expanded short-term without needing mesenchymal cells[74] (Fig. 2). In these branched organoids, the bulk of the cells were SOX9$^+$ progenitor cells; however, cells clustered at the periphery expressed the exocrine marker amylase, and cells in the centre were insulin-expressing cells, suggesting the presence of endocrine lineage cells.

The short-term expansion limitations of pancreatic progenitor organoids were addressed, and cultures derived from both human fetal pancreases at 8–11 weeks after conception and mouse embryonic pancreases at E12 to E13 were able to be cultured for over 5 months[75]. This study demonstrated that removing EGF from the culture medium drives organoids towards an endocrine phenotype while simultaneously halting their expansion. In a study published in 2024, tripotent LGR5$^+$ progenitors were identified in human fetuses (human fetal pancreas at 8–17 weeks after conception) when single LGR5$^+$ cells from embryonic pancreas were shown to be capable of generating organoids composed of ductal, acinar and endocrine lineages, which could be expanded long-term[76]. These tripotent LGR5$^+$ cells seem to be very specific to the human embryo, as they have not been found (at least not yet) in adult human tissue. The capacity for long-term expansion and differentiation of progenitors is vital for research seeking to understand pancreatic developmental stages, and their expansion ability is important to make the most efficient use of the limited nature of the sources from which they are derived (Fig. 2b).

Like PSC-derived organoids, embryonic tissue-derived organoids display an immature phenotype after differentiation. They are not perfect analogues of adult cells, as shown by, for example, the persistence of the fetal marker α-fetoprotein when compared with adult liver tissue[72]. The ability to differentiate progenitors provides an excellent model for investigating early organogenesis of the liver and pancreas, along with the mechanisms of tissue differentiation. However, the immature functional state of these embryonic cell-derived organoids reduces their ability to model the adult tissue and their use in understanding mechanisms driving adult diseases. There have been advances in culture conditions to generate more mature phenotypes, although fetal markers, albeit reduced, persisted[71]. Pancreatic progenitors possess the capacity to become multiple pancreatic cell types, and, therefore, there is potential to develop complex cultures with both exocrine and endocrine lineages and multiple hormone-secreting cell types to better model interactions during development. A greater understanding of β-cell development in these cultures might lay the foundations for improved iPSC models for patient transplants.

Liver and pancreas organoids derived from adult tissue-resident cells

Stem cells have been identified for different tissues and organs, and their capacity for replenishing damaged tissues during regeneration has been described. Unlike skin[77] or intestine[78], in which stem cell niches are well-established, in the pancreas and liver, the main drivers of homeostatic turnover and regeneration are tissue-resident differentiated cells. The culture of adult tissue-resident cells has historically proven challenging as they de-differentiate and lose their typical marker expression or show limited viability in vitro[69,79].

Adult tissue-derived liver organoids. In 2013, Huch and colleagues established protocols for generating organoids from healthy mouse liver tissue[60] (Fig. 1b). These methods were further extended to liver tissue from other species, including dogs[80] and humans, with the latter enabling the generation of organoids that model the human liver's structure and function[62]. This advance enabled researchers to study human-specific liver biology and disease. Since then, the generation of bile-derived organoids from cells extracted from bile fluid has also been described[81]. These organoids provide a unique perspective on the biliary system and its role in liver function and disease. In 2022, minor modifications to these culture conditions enabled the establishment of branching cholangiocyte organoids[82] in which ductal cells do not expand their lumen isotropically, generating a cyst, but create a tube-like morphology in culture (Fig. 1b).

In 2018, in two independent studies, organoid cultures were successfully generated from both mouse and human hepatocytes[70,83] (Fig. 1b). As hepatocyte proliferation substantially increases after physical or chemical damage in vivo, both studies approached the challenge of expanding hepatocytes in vitro by testing injury-related stimuli. Peng and colleagues showed that TNF, a pro-inflammatory cytokine critical for fetal liver growth and liver regeneration after injury, is a key driving factor for hepatocyte expansion in three-dimensional culture. ScRNAseq of these mouse hepatocyte

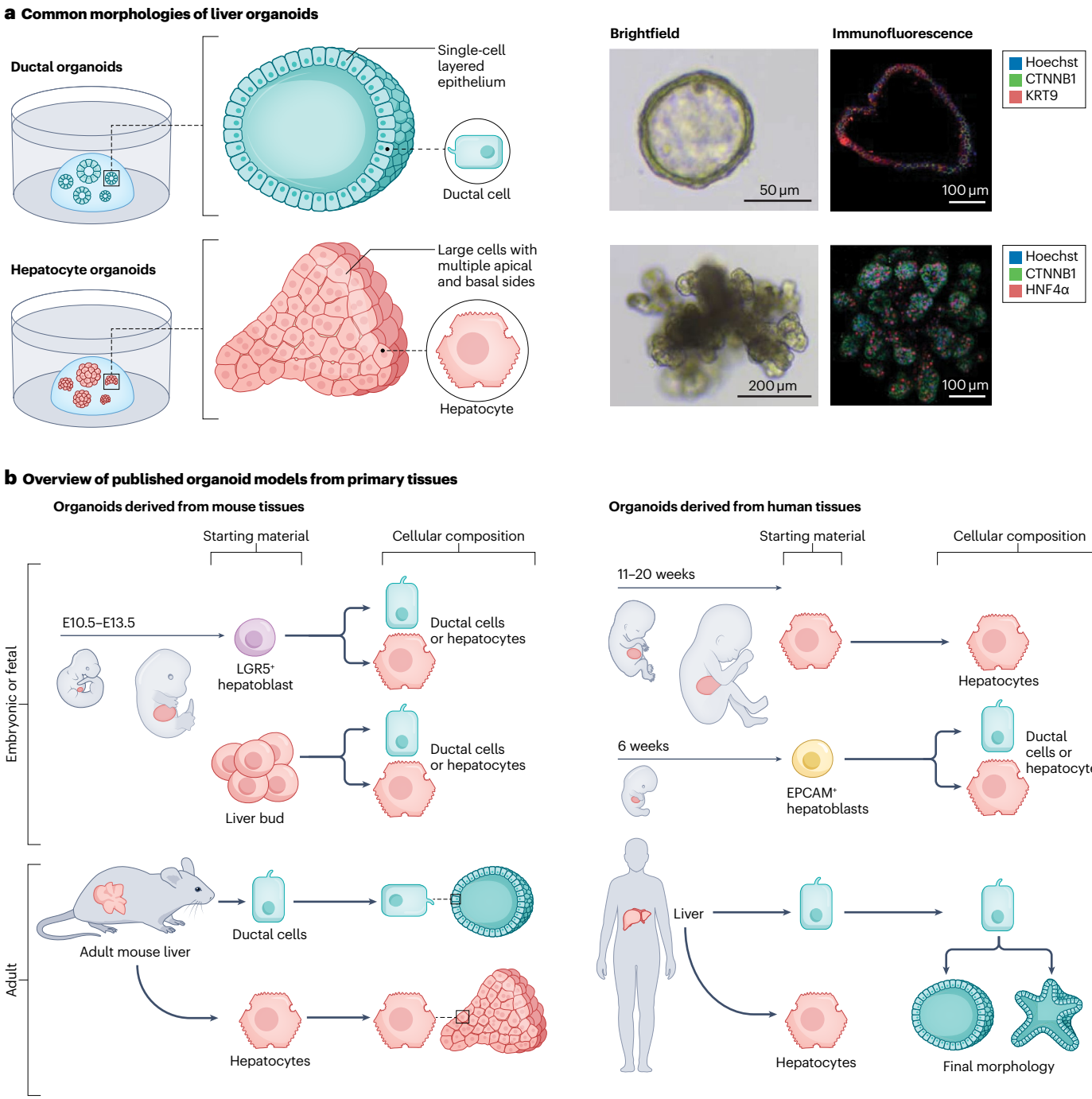

Fig. 1 | Liver organoids derived from different tissue-resident cells. **a**, Common morphologies of liver organoids derived from liver ductal cells (top left) or hepatocytes (bottom left). Ductal cells form single-cell layered epithelial organoids with usually cystic lumina, whereas hepatocyte organoids form dense, branching structures. The microscopy images on the right show mouse organoids derived from hepatoblasts, embryonic bipotent progenitors that, depending on the medium used, can be grown into either ductal or hepatocyte-like organoids. On brightfield, ductal organoids can be seen as cystic structures (top), whereas hepatocyte organoids form opaque structures (bottom). The immunofluorescence images show typical epithelial (CTNNB1), ductal or hepatocyte markers, KRT19 or HNF4α, respectively. **b**, Overview of some of the first published organoid models from primary liver epithelial cells. Organoids derived from mouse cells are shown on the left, and organoids derived from human cells on the right; organoids derived using developmental stage liver are shown at the top and organoids derived using adult liver (mouse, 8–12 weeks) at the bottom. Ductal organoids can be derived from primary ductal cells and hepatocyte organoids can be derived from primary hepatocytes. Additionally, both hepatocyte and ductal organoids can be derived from a single cell source, embryonic hepatoblasts. E, embryonic day; EPCAM, epithelial cell adhesion molecule; LGR5, leucine-rich repeat-containing receptor 5. Immunofluorescence and brightfield images were adapted with permission from ref. 72. Company of Biologists.

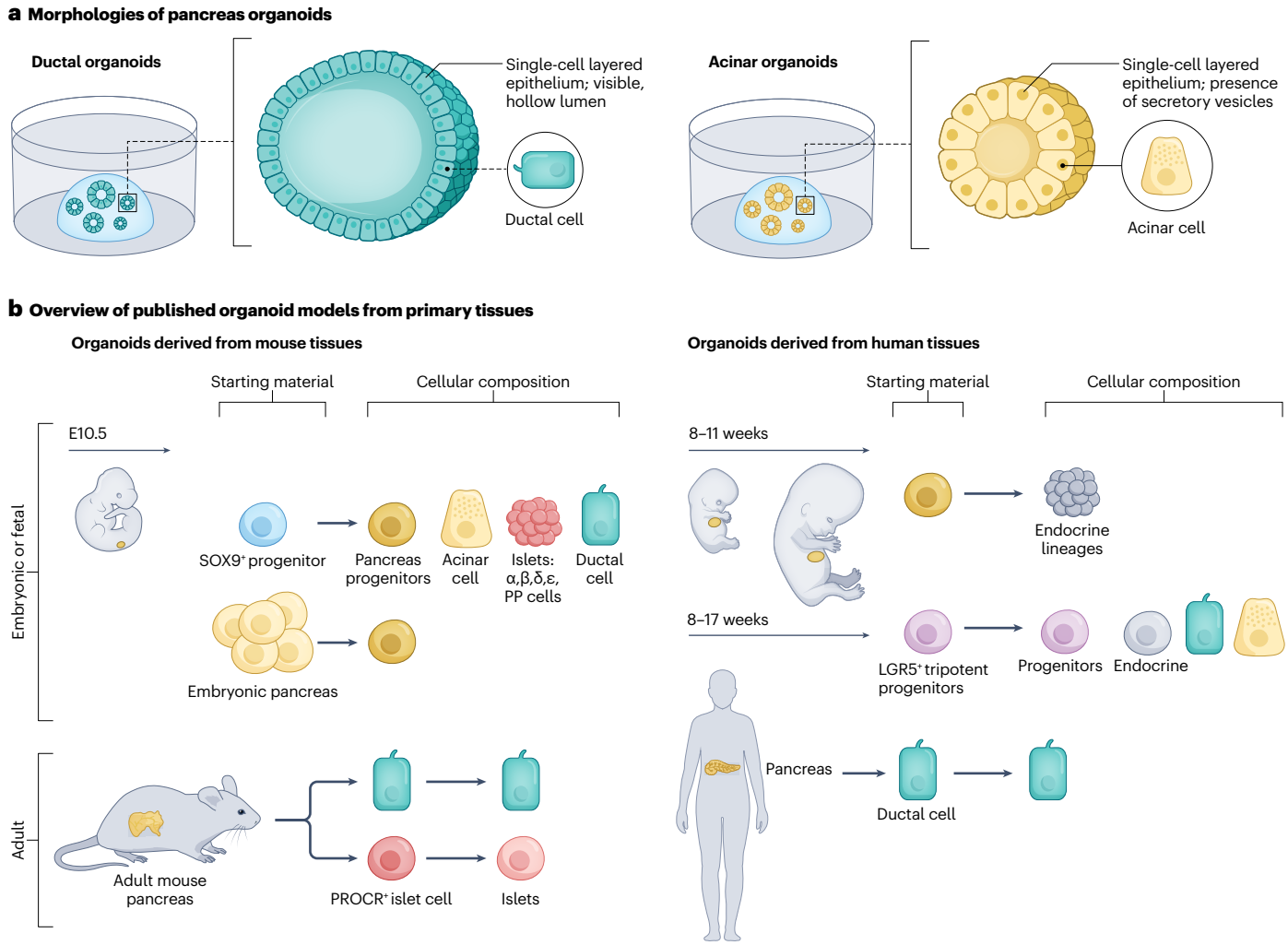

Fig. 2 | Pancreas organoids derived from different tissue-resident cells. **a**, Morphologies of pancreas organoids derived from ductal cells (left) and acinar cells (right). Ductal cells form single-cell layered epithelial organoids with a visible lumen; acinar organoids form single-cell layered epithelial organoids with a smaller lumen, hardly visible on brightfield, but with secretory vesicles present and visible. **b**, Overview of some of the first published organoid models from primary pancreatic cells. Organoids derived from mouse cells are shown on the left, and organoids derived from human cells on the right; organoids derived using developmental stage pancreas are shown at the top, organoids derived using adult pancreas at the bottom. Starting with different progenitors, organoid models of the embryonic and fetal pancreas can be made to give rise to different pancreas lineages, including ductal and acinar cells and islets (α, β, δ, ε and pancreatic polypeptide (PP) cells). Organoid modelling of the adult pancreas has been predominantly successful for ductal cells, with optimization of culture conditions to facilitate long-term expansion in chemically-defined media. Endocrine lineages were reported initially from embryonic and fetal tissue, but a method to expand islet organoids from cells isolated from adult mouse pancreas has been described recently. E, embryonic day; LGR5, leucine-rich repeat-containing receptor 5; PROCR, protein C receptor; SOX9, SRY-box transcription factor 9.

organoids revealed broad expression of hepatocyte markers, with a cluster of cycling hepatocytes expressing proliferation markers including *Mki67*, *Ccna2*, *Cdk1*, *Top2a* and *Cenpm*[83]. Upon withdrawal of expansion signals (TNF), functional genes were further upregulated, moving the transcription profile towards quiescent primary hepatocytes. Conversely, the culture conditions determined by Hu and colleagues included R-spondin (a WNT agonist) in the absence of TNF[70]. Although hepatocyte organoids displayed typical functional aspects and transcription signatures resembling those of primary hepatocytes, they also expressed fetal markers including α-fetoprotein AFP. Moreover, the organoids showed increased proliferation, which is upregulated in the liver upon partial hepatectomy, a widely used form of physical liver injury[70]. In 2025, the Huch group reported optimization of culture conditions for mouse hepatocyte organoids, which allowed the formation of a functional network of bile canaliculi with diameter and network properties akin to those in mouse liver tissue (described in more detail in the section 'Limitations and future directions')[84]. In parallel, combining WNT with either STAT3 signalling[85] or YAP activation[86] facilitates the expansion of adult human hepatocyte organoids that retain a functional bile canaliculi network and perform major metabolic functions of the liver[85] upon removal of the niche factors. Collectively, the data suggest that the mechanisms driving liver

regeneration after injury in vivo are key to achieving the expansion of hepatocytes in three-dimensional culture.

Adult tissue-derived pancreas organoids. Unlike the small intestine, in which a pool of LGR5$^+$ stem cells has been identified[78], the presence, identity or location of progenitor cells in the adult pancreas is still under investigation and is a matter of debate. Similar to the liver, the adult pancreas does not have a designated pool of active stem cells, but pancreatic cells retain plasticity, with discrepancies between human and mouse models[87–89]. Pancreas development and regeneration have been reviewed in detail elsewhere[90,91].

Initial ideas of propagating adult pancreas cells in vitro were inspired by the combination of two observations: on the one hand, the isolation of pancreas ductal cells[92] and, on the other hand, the activation of epithelial proliferation after an acute injury. Acute injury (in particular, pancreatic duct ligation[93]) results in the upregulation of WNT signalling and expression of the stem cell marker *Lgr5* in the proliferative duct compartment[61]. This observation led to the generation of adult pancreas organoids[61] by culturing duct fragments or single isolated cells in a medium containing R-spondin 1, the LGR5 ligand and co-activator of the WNT pathway[2,94,95], and several growth factors important for pancreas development, including EGF and the FGFR2 and FGFR4 ligands, essential for pancreas development[96]. Culturing mouse pancreatic epithelial cells in the same medium demonstrated that ductal cells (*Sox9*$^+$), but not acinar cells (*Ptf1a*$^+$), could self-renew long-term in vitro[61]. SOX9$^+$ ductal cells developed into hollow cystic organoids, positive for duct-specific markers such as pan-cytokeratin, mucin 1 and cytokeratin 19. These pancreas organoids did not show any signs of endocrine differentiation in vitro, but when mixed with embryonic pancreas tissue and transplanted into the kidney capsule, the cells exhibited robust endocrine differentiation[61]. Although this early work showed that pancreas organoids can be expanded from the ductal epithelium, whether distinct ductal cells have different organoid formation potential remained unresolved. In this regard, a recent study reported by Fernández and colleagues showed that the healthy ductal epithelium of the mouse pancreas exists in 15 distinct ductal cell states, which have different organoid formation capacities[97]. The researchers found a WNT-responsive ductal cell subpopulation that expressed many markers of stem and progenitor cells in other tissues, including *OLFM4*, *LY6D*, *AGR2* and the WNT-responsive genes *ASCL2*, *RNF43* and *ZNRF3*, among others, but not LGR5, highlighting the difference between the findings in embryonic human pancreas[76] and adult pancreas[97]. A very interesting finding is that these distinct ductal cell populations, when grown as organoids, show different endocrine differentiation potentials. Together, these results highlight the importance of cellular heterogeneity in dictating specific functional roles of the exocrine pancreas in health and disease.

In 2015, the Clevers laboratory adapted the mouse ductal protocol from 1961 to generate pancreas organoids from patient-resected tumours and biopsy samples by including TGFβ pathway inhibitors (A83-01 and Noggin), EGF and PGE2 (ref. 98). This protocol also hinted at the possibility of expanding healthy pancreas tissue in humans; however, unlike tumour organoids, healthy tissue-derived organoids could not be sustained long in culture. Following studies from the de Koning laboratory using human islet-depleted pancreatic tissue cultured in an EGF–Noggin–R-spondin-based medium showed that pancreatic cells would develop into budding, cauliflower-like structures[99]. Tip regions of budding structures were positive for LGR5, pancreatic progenitor markers PDX1 and SOX9. When tested for aldehyde dehydrogenase activity, as a surrogate for progenitor cell markers, cells at the tips showed high aldehyde dehydrogenase activity. In vitro, differentiation of organoids led to upregulation of the endocrine progenitor marker *NEUROG3* and β-cell marker *NKX6.1*, whereas *PDX1* and *SOX9* remained present. Only upon xenotransplantation into immunodeficient mice did de novo insulin-positive cells form[99]. In 2020, these human pancreas organoids were further optimized in the Huch laboratory establishing culture conditions with serum-free medium and chemically defined, modifiable, scalable, biomimetic hydrogel[63] (Fig. 2b). These conditions enabled the successful establishment of human pancreas organoids that would expand long-term, including from cryopreserved pancreatic tissue, and retain their genetic stability over months in vitro[63]. Subsequent studies using single-cell analysis showed that these ductal organoids were similar to the human ductal epithelium[100].

One of the major remaining challenges is to expand, retain and/or de novo form endocrine compartments in vitro. Wang and colleagues, using an approach involving overlaying cells in Matrigel (a basement membrane extract from Engelbreth–Holm–Swarm mouse sarcoma produced by Corning Life Sciences), showed that islet organoids can be generated when using mouse PROC$^+$ pancreas cells[34,101]. For that, the researchers used a *Proc1*$^+$*Gfp*$^+$ mouse reporter and sorted PROC1$^+$ cells that subsequently formed functional islet organoids in a three-dimensional culture responsive to glucose challenge[101] and able to reverse type 1 diabetes mellitus upon transplantation. A follow-up study, using single-cell ATAC sequencing and scRNAseq data in mouse and human tissues, showed that PROCR$^+$ progenitor cells might be derived from embryonic mesothelial cells, supporting a developmentally relevant function for these PROCR-like cells during in vivo pancreas development[102]. Until now, starting from exocrine ductal epithelium, complete differentiation of endocrine cells was possible only upon transplantation[61,99]. These experiments challenged the previous notion that duct cells can only be converted into insulin-producing cells in vitro upon overexpression of the β-cell-specific transcription factors MAFA, PDX1, NGN3 and PAX6 (ref. 103). Instead, these studies demonstrated that the mouse and human adult pancreatic ductal epithelial cells are not epigenetically silent but retain cellular plasticity and endocrine differentiation potential, which is regulated by the cellular microenvironment[61]. These results are in agreement with those from in vivo mouse models of pancreas duct ligation[104] and the AKITA diabetes mellitus mouse model[105], which indicated that upon substantial challenge of the pancreas endocrine tissue, the ductal epithelium can rescue the loss of β-cell mass, in a mechanism that is not yet completely understood.

Disease modelling and applications with liver and pancreas organoids

Optimally, a disease model recapitulates the genetic profile, cellular heterogeneity and tissue structure at different stages of disease and responds to stimuli in a physiological manner. In this section, we describe the modelling and use of single-cell-type (epithelial) organoids. As the generation of models that include both parenchymal and non-parenchymal compartments is still in its infancy, we address multitissue and multiorgan organoids in the 'Limitations and future directions' section of this review.

Disease modelling using liver organoids

Therapeutic applications of liver organoids have been comprehensively reviewed elsewhere[33,35,36,106]. Disease modelling of the liver requires the establishment of reproducible models of the adult and embryonic liver.

With the advance in liver organoid technologies and improvement in the architecture and function of the systems, it has been possible to start to model and understand liver disease (Table 2).

Modelling inborn genetic diseases. Development of healthy human liver organoid models by Huch and colleagues in 2015 was paralleled by establishment of the first patient-derived liver organoids, in which differentiated liver organoids derived from patients with α1-antitrypsin deficiency displayed an accumulation of A1AT protein in the liver cells, as seen in the patient biopsy samples. In the same study, in differentiated liver organoids derived from patients with Alagille syndrome, cholangiocytes failed to integrate into the organoid epithelium and were apoptotic, resembling the patients' phenotype, which shows ductopenia[62]. In a follow-up study, Wills and colleagues in 2016 applied the same protocol to generate organoids from patients with autosomal dominant polycystic liver disease, which enabled the expansion of the diseased tissue to obtain enough material for a whole-genome sequencing analysis, leading to the conclusion that in human patients, liver cysts have unique genetic makeups[107]. Importantly, these studies demonstrated the potential of the system to model monogenic liver diseases[62,107].

In parallel, several groups have now demonstrated the feasibility of generating liver organoids from iPSCs that carry mutations causing inborn liver diseases. Guan and colleagues in 2017 developed liver organoids from patient-specific iPSCs from patients with Alagille syndrome and by genetically engineering iPSCs carrying mutations in *JAG1* (ref. 108). The organoids exhibited altered morphology with fewer cholangiocytes in the duct structures. In 2023, Mun and colleagues developed a protocol for differentiating iPSCs into glycogen storage disease type 1a (GSD1a) patient-specific liver organoids that maintained higher lipid and glycogen accumulation and lactate secretion into the medium, consistent with the main disease-specific characteristics of patients with GSD1a[109]. Monogenic and inborn disease-derived models have been extensively reviewed elsewhere[110].

Collectively, these studies demonstrate that liver organoids, whether derived from iPSCs or patient tissue, serve as highly relevant platforms for studying inborn liver diseases, from biliary defects to hepatocellular dysfunction. The organoids' ability to recapitulate patient-specific phenotypes opens avenues for precision medicine, especially when combined with genome editing and drug screening technologies. However, challenges such as intrahepatic cell–cell interactions or multiorgan crosstalk result in an incomplete modelling of advanced stages the disease (for example, fibrosis or cirrhosis). As we discuss in the section 'Limitations and future directions', we envision that the increase in complexity of these liver organoid models will enable, in the near future, the development of models that will better reproduce most of the aspects of these monogenic and inborn liver diseases.

Modelling liver steatosis. Given the increasing incidence of MASLD and metabolic dysfunction-associated steatohepatitis (formerly known as non-alcoholic steatohepatitis), one of the applications for hepatocyte organoids has also been to model diseases involving lipid metabolism, namely steatosis and steatohepatitis. In 2019, Ouchi and colleagues reported the first attempts to model and understand steatosis using iPSC human liver organoids[111]. The authors generated human liver organoid models containing epithelial and stromal lineages from PSCs and treated them with free fatty acids to mimic key stages of steatohepatitis: steatosis, inflammation and fibrosis[111]. They also developed organoids from patients with Wolman disease[111] and showed that these phenocopied severe steatohepatitis. Although FGF19 showed promising antisteatotic activity, their findings also indicated that to model steatosis, it is necessary to generate hepatocyte organoids with mature hepatocytes[111]. In a study in 2022 by Belenguer and colleagues, knockout of *Rnf43–Znrf3ko*$^{/ko}$ (both protein products act as negative regulators of WNT signalling) in adult mouse hepatocyte and hepatoblast organoids resulted in elevation of lipid droplet accumulation, compared with the levels in *Rnf43–Znrf3flox*$^{/flox}$ controls[112]. This phenotype was rescued with WNT inhibitors, indicating the importance of WNT signalling in the development of steatohepatitis. This research showed the cell-autonomous effect of RNF43 and ZNRF3 metabolism of lipids in hepatocytes, which eventually leads to hepatocellular carcinoma (HCC)[112] (Fig. 3a). Similarly, Hendriks and colleagues used CRISPR-engineered human fetal hepatocyte organoids to model steatosis and perform a drug screen[113]. Through prime editing of patient-specific SNPs — *PNPLA3I148M*, or CRISPR knockout *APOB* or *MTTP* — hepatocyte organoids presented a steatotic phenotype. These steatosis-like hepatocyte organoids (genetically derived or induced by free fatty acid) were used to find inhibitors of ACC, FAS and DGAT2 as drugs that effectively prevent lipid accumulation[113]. However, MASLD is a multiorgan disease, and although these organoid models have proven useful to underscore some of the genetic determinants, cell-autonomous and non-autonomous mechanisms[111–113], they fail to replicate the systemic aspects of the disease. Similarly, they are limited in their ability to reproduce the effect of metabolic comorbidities, which highlights the importance of combining these models with other systems (co-cultures, microfluidics) and in vivo models to gain a comprehensive understanding of MASLD.

Modelling cholestasis and other complex liver conditions. Cholestasis is a condition in which bile flow from the liver is reduced or blocked. This condition can lead to the accumulation of bile acids in the liver parenchyma and, if severe, also in the bloodstream, causing liver damage and symptoms such as jaundice, among others. Cholestasis can result from liver diseases, bile duct obstructions (for example, gallstones or tumours), genetic disorders or drug-induced liver injury[114–116]. Modelling cholestatic liver disease in hepatocyte organoids has proved challenging, given that bile canaliculi already presented cholestatic features such as dilation and a non-physiological diameter. Using the optimized culture conditions for mouse hepatocyte organoids, it was shown that the genetic form of cholestasis induced by *Mdr2*$^{-/-}$ deficiency can be efficiently reproduced in vitro in hepatocyte organoids. By culturing *Mdr2*$^{-/-}$ hepatocytes as hepatocyte organoids, the researchers reproduced the cholestatic features of the mutant mouse tissue, including apical bulkheads and rosette-like lumina formation in vitro[84]. Similarly, in a 2023 study, culturing hepatocyte organoids with a three-dimensional bile canalicular network enabled the modelling of the acute cholestatic injury induced by treatment with high concentrations of the secondary bile acid, deoxycholic acid. Deoxycholic acid-treated hepatocyte organoids showed bile canaliculi dilation and several features of cholestasis, including dilated bile canaliculi with apical bulkheads and rosette-like lumina[117] (Fig. 3a).

Cholangiocyte organoids derived from biliary atresia patients showed delayed epithelial development, impaired permeability, decreased identity markers and abnormal polarity, akin to the findings in patient tissue. This phenotype was reversed in biliary atresia-derived cholangiocyte organoids by inducing differentiation through EGF and FGF2 signalling, implying that signalling that guides cholangiocyte differentiation is impaired in patients with biliary atresia[118] (Fig. 3b). Similarly, bile-derived organoids from patients with primary sclerosing cholangitis show increased immune modulator markers compared

Table 2 | Comparative list of developments in liver and pancreas organoid disease models, including their source, composition and the disease state they recapitulate

Tissue modelled	Starting material	Disease category	Disease type	Organoid composition	Species	Derived from	Ref.
Intrahepatic cells (liver)	Tissue-resident cells	Inborn diseases	A1AT deficiency	Hepatocyte-like cells	Human	A1AT-deficient patient-derived ductal cells	Huch et al. (2015)[62]
			Wilson disease	Ductal cells	Dog	COMMD1-deficient ductal cells	Nantasanti et al. (2015)[80]
		Acquired diseases	HCC CC CHC	Cancer hepatocytes, ductal cells or mixed type	Human	Tumour resections	Broutier et al. (2017)[122]
			HCC CC	Cancer hepatocytes and ductal cells	Human	Tumour needle biopsy sample	Nuciforo et al. (2018)[123]
			HCC CC CHC	Cancer hepatocytes, ductal cells or mixed type	Human	Tumour resections	Yang et al. (2024)[125]
			CC with *BAP1* mutation	Ductal cells	Human	Adult healthy ducts	Artegiani et al. (2019)[127]
			SARS-CoV-2 infection	Ductal cells	Human	Adult healthy ducts	Zhao et al. (2020)[120]
			HBV infection	Ductal cells	Human	Ductal cells from patients with HBV infection	De Crignis et al. (2021)[124]
			Steatosis	Hepatocytes	Mouse	Adult mutant hepatocytes (*Rnf43* and *Znrf3*)	Belenguer et al. (2022)[112]
			Steatosis	Hepatocytes	Human	Fetal hepatocytes	Hendriks et al. (2023)[113]
			Cholestasis	Hepatocytes	Mouse	Adult healthy hepatocytes	Mayer et al. (2023)[117]
			Biliary atresia	Ductal cells	Human	Liver biopsy sample	Amarachintha et al. (2022)[118]
			Primary sclerosing cholangitis	Ductal cells	Human	Bile from patients with primary sclerosing cholangitis	Soroka et al. (2019)[81]
			Cholestasis	Hepatocytes	Mouse	Adult mutant hepatocytes (*Mdr2*)	Dowbaj et al. (2025)[84]
			GSD1a	Ductal cells and hepatocyte-like cells	Human	iPSCs	Mun et al. (2023)[109]
	PSCs	Inborn diseases	Alagille syndrome	Ductal cells	Human	iPSCs	Guan et al. (2017)[108]
			Cystic fibrosis	Ductal cells	Human	Mutant iPSCs — *CFTR* (ΔF508)	Ogawa et al. (2015)[41] Sampaziotis et al. (2015)[42]
		Acquired diseases	HBV infection	Ductal cells	Human	iPSCs	Nie et al. (2018)[119]
			Alcoholic fatty liver	Ductal cells Hepatocytes	Human	ESCs	Wang et al. (2019)[44]
			Steatohepatitis	Ductal cells and hepatocyte-like cells	Human	iPSCs	Ouchi et al. (2019)[111]
Pancreatic cells	Tissue-resident cells	Acquired diseases	PDAC	Ductal cells	Human	Tumour resections	Boj et al. (2015)[98]
			PDAC	Ductal cells	Human	Tumour fine-needle biopsy sample	Tiriac et al. (2018)[132]
			PDAC	Ductal cells	Human	Tumour resections	Seino et al. (2018)[135]
			PDAC, IPMN	Ductal cells	Human	Tumour resections	Georgakopoulos et al. (2020)[63]
			ADM	Ductal cells	Mouse	Acinar cells from genetically engineered mice	Bhalerao et al. (2023)[133]

Table 2 (continued) | Comparative list of developments in liver and pancreas organoid disease models, including their source, composition and the disease state they recapitulate

Tissue modelled	Starting material	Disease category	Disease type	Organoid composition	Species	Derived from	Ref.
Pancreatic cells (continued)			PASC	Ductal cells	Human	Tumour resections and endoscopic ultrasound-guided FNA	Tamagawa et al. (2024)[136]
			PDAC	Ductal cells	Mouse	Primary cells from genetically engineered PDAC mice	Papargyriou et al. (2024)[137]
	PSCs	Inborn diseases	Cystic fibrosis	Ductal cells	Human	iPSCs	Hohwieler et al. (2017)[139]
		Acquired diseases	PDAC	PDAC	Human	ESCs	Huang et al. (2015)[50]
			Diabetes mellitus	Islet-like organoids	Human	Primary islet cells	Ilegems et al. (2022)[152]
Multitissue organoid	Tissue-resident cells	Acquired diseases	Biliary fibrosis	Hepatocytes, ductal cells and portal fibroblasts	Mouse	Adult healthy hepatocytes, ductal cells and portal fibroblasts	Dowbaj et al. (2025)[84]
			Biliary fibrosis	Hepatocytes, ductal cells and portal fibroblasts	Human	Patient-derived healthy hepatocytes, ductal cells and portal fibroblasts	Yuan et al. (2025)[86]
	PSCs	Vascularized macrophage-islet organoids	SARS-CoV-2 and coxsackievirus B4 infection	Individually hiPSC-derived islet cells, macrophages and endothelial cells	Human	iPSCs	Yang et al. (2024)[177]
		Midgestational fetal liver organoids	Fibrosis	Hepatocyte-like cells, liver endothelium, liver mesenchyme from septum transverse	Human	iPSCs	Tadokoro et al. (2024)[169]

ADM, acinar to ductal metaplasia; CC, cholangiocarcinoma; CHC, combined HCC–CC; ESC, embryonic stem cell; FNA, fine-needle aspiration; GSD1a, glycogen storage disease type 1a; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; hiPSC, human induced PSC; IPMN, intraductal papillary mucinous neoplasm; iPSC, induced PSC; PASC, pancreatic adenosquamous carcinoma; PDAC, pancreatic ductal adenocarcinoma; PSC, pluripotent stem cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with the organoids derived from healthy individuals[81]. In addition, current studies have shown that liver organoids can be used to investigate aspects of infectious diseases, including virus–host interactions during hepatitis B virus (HBV) infection[119]. The liver ductal organoids were used during the 2019 coronavirus disease pandemic to investigate the causes of liver failure in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[120] (Fig. 3c). These works led to the discovery that the virus infects the ductal epithelium, causing direct damage and subsequent liver injury[121]. It is unknown whether hepatocytes and/or other cell types in the liver tissue are affected as well. Complex in vitro models that recapitulate the intricate architecture of liver tissue are starting to emerge (see the section 'Limitations and future directions'). However, liver organoids are still limited in both cellular diversity[84] and maturity[45], which poses a great challenge for the future study of not only these infectious diseases but also all other liver conditions, including monogenic disorders, in which the impact of epithelial damage on the surrounding niche cells cannot be adequately investigated.

Liver organoids derived from cancer tissue. Liver cancers are categorized by the cell type they are derived from: HCC, cholangiocarcinoma (cholangiocytes) and combined HCC–cholangiocarcinoma tumours (Fig. 3d). In a 2017 study by Broutier and colleagues, liver cancer organoids derived from patient biopsies of all the tumour subtypes were successfully cultured long-term while maintaining the mutation and expression profile of the original tumours. In this study, the investigators identified ERK inhibition as an effective drug for the subset of patient-derived HCC and cholangiocarcinoma organoids[122]. Similarly, in a 2018 study by Nuciforo and colleagues, human liver cancer organoids were derived from needle biopsy samples (HCC and cholangiocarcinoma) from patients and showed long-term expansion potential and mutational stability. Employing the organoids, these investigators demonstrated patient-specific sensitivity to sorafenib[123]. In a study by De Crignis and colleagues, HBV-infected organoids were found to have an aberrant early cancer gene signature, which clustered with the gene signature in tissue from individuals with HCC, providing an invaluable tool for the study and surveillance of patients with HBV infection[124] (Fig. 3c). These systems promise to uncover patient-specific therapeutic vulnerabilities for all subtypes of liver cancer.

As the first proof-of-concept papers, these studies showed that establishing cancer organoids from liver tumours and using them for drug screening is feasible. However, the extreme heterogeneity of liver cancer subtypes posed the challenge that only liver cancer from undifferentiated tumours was expandable. By expanding on these findings in larger cohorts of patients, in 2024, Yang and colleagues developed a living biobank of more than 140 patients, which confirmed that liver cancer organoids can be used to identify multigene expression signatures and predict drug responses[125]. The predictive value of liver cancer organoid models for informing patient care is lacking, and comprehensive, multicentre collaborative studies will be required to evaluate their clinical relevance as detailed previously[126]. Cancer gene function can also be investigated using healthy liver organoids via targeted gene editing techniques such as CRISPR–Cas9, as demonstrated by the regulation of chromatic accessibility tumour suppressor BAP1 in human cholangiocyte organoids[127].

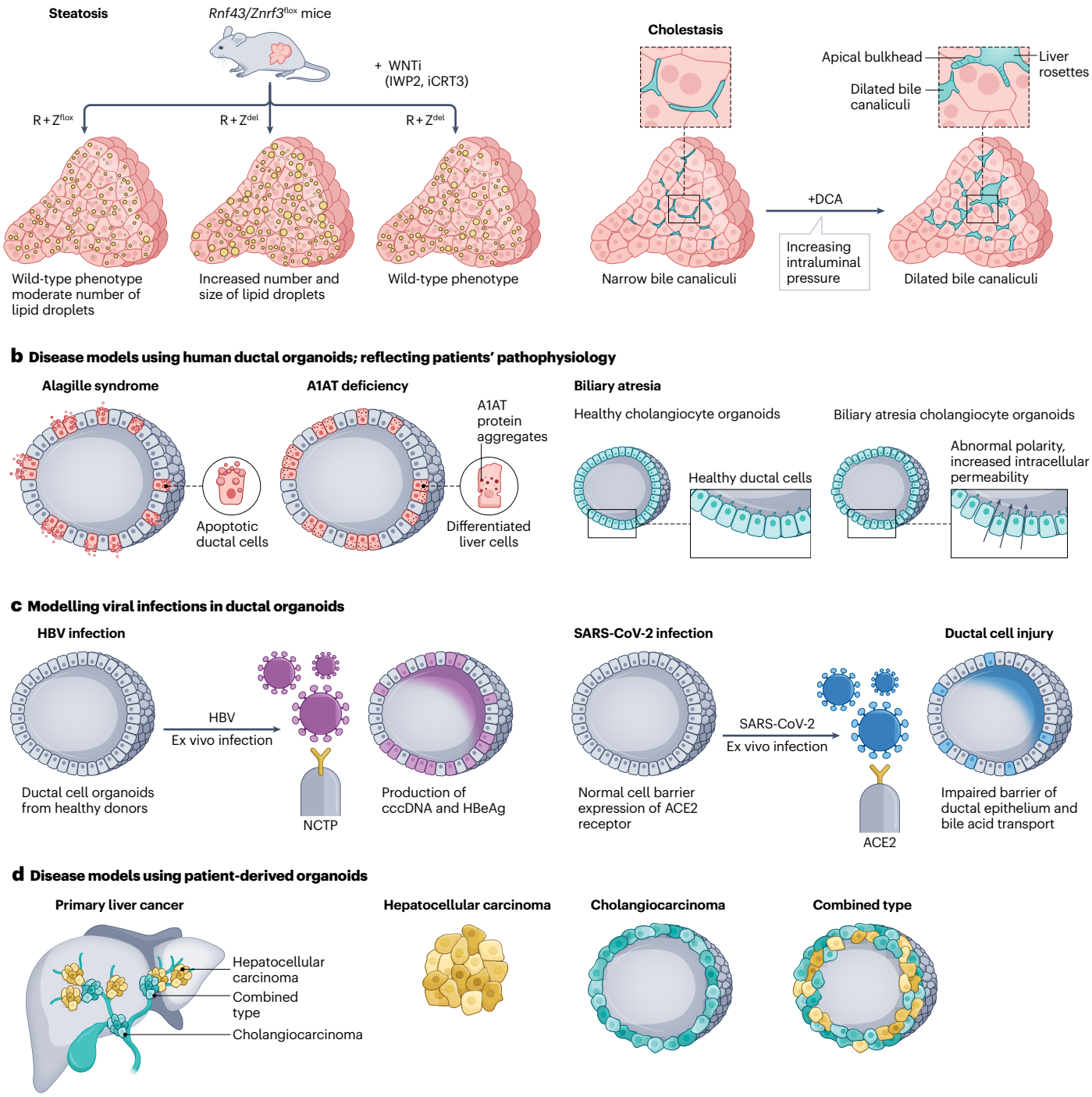

Fig. 3 | Disease modelling using liver organoids. Organoids can be used to model aspects of disease using either patient cells (part **b**) or healthy cells as starting material to generate organoids, and to model disease responses to small-molecule administration (part **a**) or viral infection (part **c**). Organoids can also be derived from patient tissues to model different types of cancer in which the tumouroids recapitulate the genetic and phenotypic traits of the tumour of origin (part **d**). A1AT, α1-antitrypsin deficiency; ACE2, angiotensin-converting enzyme 2; cccDNA, covalently closed circular DNA; DCA, dichloroacetic acid; del, deletion; HBeAg, HBV early antigen; HBV, hepatitis B virus; NCTP, sodium taurocholate co-transporting polypeptide; R, ring finger protein 43 (RNF43); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WNTi, Wingless-related integration site inhibitor; Z, zinc and ring finger 3 (ZNRF3).

In summary, the liver is a complex organ that maintains homeostasis by interacting with epithelial and non-epithelial cells. Many aspects of the diseases can be modelled using only one epithelial cell type. However, to understand the complexity of the disease progression, researchers need to introduce non-epithelial niche cell types to understand their role in the dynamics of liver pathologies. Multi-cell-type liver organoids are discussed in the section 'Limitations and future directions'.

Disease modelling using pancreas organoids

Diseases in the pancreas encompass both the exocrine and the endocrine parts of the pancreas. The most common endocrine disorder of the pancreas is diabetes mellitus, which includes type 1 and type 2 diabetes. The exocrine pancreas has been mostly studied in the context of carcinogenesis of acinar and ductal cells and cystic fibrosis. In this part, we focus on modelling these diseases.

Exocrine pancreas models of cancer. Pancreatic cancer is predicted to be the second leading cause of cancer-related deaths in the USA by 2040. Concerningly high mortality rates (88–89% over 5 years)[128,129], asymptomatic precancerous lesions and lack of effective treatment demand a better understanding of pancreatic cancer pathogenesis. Broadly, pancreatic tumours can be divided into exocrine tumours (arising from exocrine pancreas cells) and neuroendocrine tumours (originating from endocrine pancreas)[130,131]. Exocrine tumours, more specifically PDACs, comprise over 85% of pancreatic cancers, and 80% of patients are diagnosed only in the advanced stages[128,129]. Thus, most pancreas organoid models focus on recapitulating PDACs.

The establishment of PDAC organoids from biopsy samples[50,63,98,132], and iPSCs[50] has been demonstrated. The first PDAC organoids were developed from adult mouse and human tissue using a Matrigel dome organoid approach[98]. PDACs were established from healthy or low-grade mouse pancreatic intraepithelial neoplasia (PanIN) tissue by seeding small interlobular ducts. Upon orthotropic transplantation of pancreas organoids derived from PanINs, transplants developed early PanIN-like structures, showing the utility of the system to model early pancreatic neoplasia. Transcriptomic and proteomic analysis of normal and tumour ductal organoids revealed nucleoporin upregulation in mouse tumour organoids, highlighting them as potential drivers of pancreatic cancer progression. Human PDAC organoids were derived from resected tumours and biopsy samples (through fine-needle aspiration) and, upon transplantation, showed a spectrum of PanIN progression and invasive and metastatic carcinoma[98] (Fig. 4b).

Differentiating pancreas progenitors towards acinar or ductal fate enabled researchers to explore the effect of the known mutations on specific cell types of the exocrine pancreas. Huang and colleagues demonstrated that the *GNAS*^{R201C} mutation was more effective in cystic expansion (more reminiscent of ductal than acinar organoids), whereas *KRAS*^{G12D}, but not *GNAS*^{R201C}, induced acinar-to-ductal metaplasia in acinar organoids (Fig. 4a). Additionally, *KRAS*^{G12D} was more effective in modelling cancer in vivo when expressed in acinar than in ductal organoids[53]. Similarly, in PDLOs derived from human PSCs (both hESC and hiPSC), *KRAS*^{G12D} showed a similar pattern: *GNAS*^{R201C}-overexpressing PDLOs formed large cysts reminiscent of intraductal papillary mucinous neoplasms in tissue, whereas *KRAS*^{G12D} induced an epithelial–mesenchymal transition phenotype[51]. Similarly, Bhalereo and colleagues used pancreas organoids to elucidate the role of ST6GAL1 in acinar to ductal metaplasia[133].

Pancreatic cancer organoids have also been used to investigate the effect of niche signalling and niche cells in cancer initiation and progression. In that regard, generation of PDAC organoids using an air–liquid interface approach enabled the preservation of the tumour stroma, specifically the tumour-associated fibroblasts[134]. Additionally, using patient-derived cancer organoids, Seino and colleagues showed that PDAC organoids can be classified into three subcategories according to their dependence on WNT signalling. Although niche cancer-associated fibroblasts supported the growth of a subtype of PDAC organoids that did not secrete WNT and were WNT-dependent, *GATA6* knockdown enabled the tumour cells to gain WNT independence[135]. In a following study, the same researchers used pancreas adenosquamous carcinoma organoids to identify *TP63* as a driver for ductal-to-squamous reprogramming. Mechanistically, this was related to PRC2 complex and H3K27me3-mediated suppression of the ductal lineage genes and upregulation of squamous lineage genes under hypoxia and WNT-deprived conditions[136] (Fig. 4c). Both studies exemplify how environmental context is crucial for the evolution of pancreatic cancer subtypes[135,136]. Current studies have shown the necessity to understand the phenotypic diversity of PDAC subtypes in PDACs by using branching pancreas organoids embedded in collagen gels[137]. Phenotypic variability of the organoids recapitulated structurally and functionally PDAC human and mouse tissue subtypes, enabling the understanding of phenotype-specific vulnerabilities and targeted therapeutic strategies, including reduction of intratumoural heterogeneity. This approach helped researchers to understand how epithelial-to-mesenchymal plasticity in PDACs influences tumour adaptability to conventional treatments[137].

Modelling inborn genetic diseases. A common disease of the exocrine pancreas is cystic fibrosis, which markedly affects the pancreas due to mutations in the *CFTR* gene, leading to defective ion transport and thick mucus production. This abnormal mucus blocks the pancreatic ducts, causing damage and functional impairments[138]. Human pluripotent-derived organoids containing acinar-like and ductal-like cells have been used to model cystic fibrosis by activating the chloride channel with cAMP activation[139]. Additionally, pig cystic fibrosis-derived pancreatic duct organoids showed unresponsiveness to forskolin-induced swelling and showed that chloride but not bicarbonate ions drive the swelling phenotype in non-cystic fibrosis organoids[140].

In summary, current and emerging models of diseases of the exocrine pancreas have led to substantial advances in understanding the pathogenesis. Despite this, models of more complex diseases of the exocrine pancreas are still lacking; for example, models of acute and chronic pancreatitis. Pancreatitis involves complex interactions between acinar, ductal and immune cells. Current pancreas organoid systems lack sufficient cellular diversity to fully reconstitute these heterotypic interactions. Moving forward, including a more complex niche environment will be crucial to understanding the modulation of the diseases.

Organoid disease models of endocrine pancreas. Current studies have investigated multiple successful directed differentiation protocols from PSCs to all the pancreatic endocrine lineages, which can be used to treat diabetic animal models[141–144]. Furthermore, there have been promising results in the clinical trial of treating type 1 diabetes with chemically-induced iPSC-derived islets[145]. The chemically-induced iPSC-derived islets were transplanted beneath the abdominal anterior

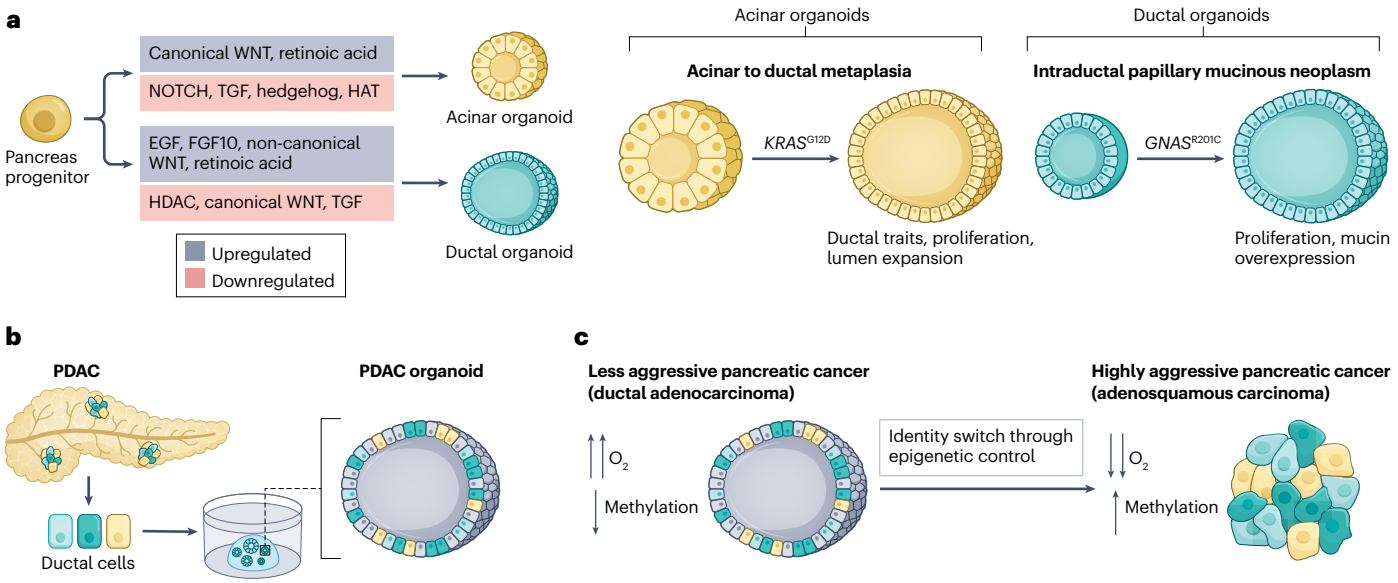

Fig. 4 | Disease modelling using pancreas organoids. Pancreas organoids have mostly been used for modelling and studying pancreatic cancer. Pancreatic cancer organoids can be made by introducing mutations into healthy acinar or ductal organoids (part **a**). Organoids can also be derived from patient biopsy samples to model pancreatic ductal adenocarcinoma (PDAC), in which the PDAC organoids recapitulate the genetic and phenotypic traits of the tumour of origin (part **b**). Cancer organoids have also been used to reveal how changes in the microenvironment can lead to the development of a more aggressive form of cancer through epigenetic control (part **c**). EGF, epidermal growth factor; FGF10, fibroblast growth factor 10; HAT, histone acetyltransferase; HDAC, histone deacetylase; NOTCH, neurogenic locus notch homologue protein 1; TGF, transforming growth factor.

rectus sheet in a patient with type 1 diabetes, and insulin independence was restored 75 days after transplantation[145]. This clinical trial represents a breakthrough in the field of stem cell therapy.

Non-conventional therapies have been used to cure diabetic mouse models, such as an optogenetic system to induce insulin secretion in hiPSC-derived pancreatic islet-like organoids[146] and induce c-peptide in the diabetic mice. In 2023, Huang and colleagues reported the differentiation of human gastric stem cells into pancreatic islet-like organoids encompassing gastric insulin-secreting cells, which, when transplanted, restored glucose levels in diabetic mice[147].

However, moving forward in the pancreas organoid field, generation of functionally mature three-dimensional pancreatic islet organoids is a necessity to understand disease mechanisms. Current advances in the islet organoid field have enabled disease modelling of some aspects of the pathogenesis of the endocrine pancreas[101,148,149]. For example, three-dimensional islet in vitro systems have been used to understand the SARS-CoV-2 infection of the endocrine pancreas. Using human ESC-derived pancreas islet three-dimensional systems, FGF7 has been shown to induce ACE2 expression in β-cells and to increase their susceptibility to SARS-CoV-2 virus[150]. Maxwell and colleagues successfully corrected the mutation in the *WFS1* gene with CRISPR–Cas9 in patient fibroblast-derived PSCs and differentiated them into pancreatic islet cell clusters. β-Cells with the corrected mutation in the *WFS1* gene produced insulin and restored glucose serum levels upon transplantation in diabetic mice[151]. Additionally, in human islet clusters, HIF1α inhibitor PX-478 improved glucose-induced insulin secretion, suggesting its antidiabetic role in metabolic overload[152].

In summary, organoid disease models of the endocrine pancreas are at an early stage but are advancing, enabling several successful cell therapy attempts in mouse models. However, to understand the origins and cell dynamics in disease progression, reliable models are needed that faithfully recapitulate tissue architecture and function. Increasingly complex systems encompassing the exocrine and endocrine pancreas would enable researchers to understand feedback loops between these two distinct parts of the organ. Finally, organoids containing multiple cell types, as well as multi-organ in vitro systems are necessary to understand complex diseases of the endocrine pancreas, as these pathologies involve interplay among several organs in the body.

Limitations and future directions

It has been shown that liver and pancreas organoids derived from tissue-resident progenitors maintain genetic stability in vitro and in vivo with the capacity for long-term expansion[62,63]. However, cells within organoids often represent immature states and do not fully differentiate into their adult counterparts from native tissue, challenging the usability of organoid models to understand adult liver and pancreas biology in vitro. The ideal (disease) organoid model should recapitulate the structure and genetic profile of the tissue under study, represent the heterogeneity and different stages of the disease, and respond to stimuli in a physiological manner. Moreover, from a practical point of view, this model needs to be feasible, reproducible, easy to maintain and inexpensive.

Over the past decade, much effort has been put into establishing, optimizing and benchmarking organoids to healthy and diseased tissues. It has been stated that organoids 'hold great promise' because of adequate three-dimensional spatial organization, similar to tissue, but they are yet to be used for discoveries. Here, we address some of the outstanding questions in the field of organoid research and suggest directions towards overcoming these challenges.

Standardization

Given the number of different isolation and culture protocols readily available, researchers should consider standardizing culturing protocols for the reproducibility of research. Two-dimensional culture systems, even if more simple, have advantages and have led to most of our current in vitro discoveries related to cell biology. What made these two-dimensional cell culture systems particularly useful is the fact that laboratories worldwide accepted the same culture protocols and a relatively simple and affordable media composition. This standardization eases the translatability of observations between research groups and enables the continuation of research. However, standardization of organoid cultures across laboratories and how to benchmark them to the respective tissues can be a challenge. In this regard, the 2023 International Society for Stem Cell Research guidelines on how to establish, maintain, characterize, authenticate and preserve stem cell and organoid cultures provide a new framework of recommendations for improving the rigour and reproducibility of these models[153]. Besides standardizing the technical protocols between research groups, the broad scientific community has already made an important step towards standardizing the definition and nomenclature for a subset of organoid types, which include hepatic, pancreas and biliary organoids[4]. As the organoid community continues to grow, there is a need for consistent nomenclature and precise language, enabling reproducibility and scientific progress[153].

Improving the accuracy of epithelial organoids by recapitulating tissue architecture

Three-dimensionality and physiological relevance. Do we, the investigators, use the three-dimensionality of organoid systems to their full potential, or can we do better? Organoids are, by definition, three-dimensional, but not all systems use this opportunity to recapitulate the three-dimensional architecture of the native tissue. Three-dimensionality is important when modelling diseases with phenotypes reflected on small, subcellular or intercellular scales. For example, we cannot model the cholestatic response in hepatocyte organoids if adjacent hepatocytes do not form narrow and interconnected bile canaliculi; that is, we cannot model cholestasis with models that are cholestatic at baseline. On the other hand, modelling the 'physiological' (that is, the branched lumen) or 'non-physiological' (that is, the cystic lumen) in pancreatic epithelial organoids, enabled the understanding of the interplay of multiple processes (cell division, cell organization and pressure), which are altered in the disease state[145].

Liver examples. One of the important aspects of any epithelial cell type is polarity, and the lumen that epithelial cells form together. For example, one of the remaining main criticisms of cholangiocyte organoids is structure-related. These organoids have isotropic growth, leading to a spherical hollow lumen instead of the branched tubular lumen formed by cholangiocytes in tissue[60,62]. From an experimental viewpoint, the closed cystic anatomy prevents access to the apical surface, limiting studies using intraluminal stimuli. A 2024 study addressed these limitations by creating functional tubular biliary organoids using a hydrogel-based organoid-on-a-chip approach. The anatomy of these organoids was reminiscent of the intrahepatic biliary tree and was perfusable, making it amenable to physical and chemical injuries from the apical surface[154].

As organoids are three-dimensional in vitro representations of organ structure and function, achieving physiological tissue architecture should be paramount to advance the complexity and physiological relevance of in vitro models. A 2025 study reported the generation of the first in vitro culture system that recapitulates complex hepatocyte polarity and bile canaliculi network properties in three dimensions[84]. Based on quantitative bioimage analysis of canalicular properties (for example, diameter and connectivity), culture conditions that resemble the geometrical features of canaliculi in the homeostatic liver tissue were selected. Thus, this study — by using an approach focusing on structure first — demonstrated that solely focusing on cell-specific markers (for example, transcription factors such as HNF4α for hepatocytes or SOX9 for cholangiocytes) or recapitulating essential cellular function (for example, albumin or bile acid secretion) is not sufficient. Achieving physiological tissue architecture is critical to advancing the complexity and physiological relevance of the in vitro models.

Despite advances in hepatocyte organoid models, replicating liver metabolic zonation remains challenging due to the complexity of spatial biochemical gradients and cellular interactions that define the portal–central vein axis and, consequently, compartmentalization of hepatocyte functions[155–157]. Traditional Matrigel-embedded organoid systems lack the ability to mimic these gradients, resulting in homogeneous hepatocyte populations that fail to capture zonated gene expression patterns and metabolic compartmentalization.

A 2024 study demonstrated a promising approach that was an alternative to tissue engineering to achieve zonation in hepatocyte organoids by co-culturing human ESC-derived hepatocytes with zonated human ESC-derived liver sinusoidal endothelial cells. Co-culture with liver sinusoidal endothelial cells of the pericentral or periportal regions modulated WNT2 signalling, inducing pericentral or periportal functional identities in hepatocyte organoids[158]. This co-culture system showed the utility of including non-parenchymal niche cells in modulating the metabolic activities of parenchymal cells. In 2025, Al Reza of the Takebe group reported the generation of multizonal human liver organoid (mZ-HLO) from hiPSC by coculturing ascorbate preconditioned zone 1-like and bilirubin-preconditioned zone 3-like hepatocyte-like cells. mZ-HLOs were transplanted into immunodeficient rats with bile duct ligation, where they outperformed single-zone control organoids in improving hyperammonaemia and hyperbilirubinaemia and enhanced rat survival[159]. Similarly, Mallanna and colleagues showed that combining human hepatocytes with dermal fibroblasts facilitates engraftment after xenotransplantation into a chronic liver injury model, leading to improved survival[160].

Pancreas examples. A study using freshly isolated embryonic pancreas epithelial cells demonstrated how the interplay between cell proliferation rate and pressure affects lumen morphology, from a 'cyst-like' lumen to 'complex' morphology with higher lumen number and occupancy[161]. By manipulating proliferation and lumen pressure (via epithelial permeability) in silico and in vitro, the researchers altered the lumen's morphological trajectories during culture growth. When the cells proliferated, they rearranged within organoids, which led to a change in epithelium permeability.

This reductionist system has multiple implications. The finely tuned balance between cell proliferation, epithelial permeability and lumen pressure could be used to understand lumen morphological changes during disease in the pancreas (cystic fibrosis or cysts in pancreatic cancers) and other organs with narrow interconnected ducts, such as the bile duct in the liver.

Advancing multilineage complexity

Organoids are reductionist models and lack many of the differentiated cell types present in the organ of interest. Although this can represent a limitation in some circumstances, it is also a benefit in enabling investigation of specific cell–cell interactions without confounding signals from other cells. On the other hand, multilineage organoid co-cultures have the promise to mimic cell–cell interactions that occur under physiological and pathophysiological conditions more closely. To represent the subregion or the niche cells encountered in an organ, the interactions between different cell populations should be recapitulated as closely as possible.

Organ function depends on multicellular interactions that organize the architecture of the organ. Thus, to recapitulate cell–cell interactions meaningfully, in vitro systems should mimic specific aspects of the tissue architecture formed for the given cell types. Mechanisms by which intercellular interactions form the architecture and what happens when they fail during disease are yet to be understood, leading to high demand for complex co-cultures for disease modelling and drug testing. Frequently, the onset of disease is initiated by one cell type. Still, throughout the disease, the major clinically known phenotype might result from the activity of another cell type in its immediate niche. Because most state-of-the-art organoids are monocultures, there is a lack of systems to mechanistically dissect how specific interactions affect pathogenesis. This is why, if possible, we should consider adding niche cells when developing models for complex diseases. However, reproducibility in multicellular and multitissue organoid systems decreases compared with that in simple organoids, as it is challenging to coordinate the proliferation and differentiation of multiple cell types[4], highlighting the need for new strategies to create multilineage liver and pancreas cultures. Some of the current strategies for multilineage cultures are discussed below.

Separated compartments. Organ-on-chip technology is used to overcome the challenges of coordinating the proliferation and differentiation of multiple cell types. By using microfabrication, it is possible to create physically separated compartments with properties suitable for the cells of interest. Communication via secreted factors is enabled through porous membranes, but direct cell–cell contacts are limited by design. Additionally, liver-on-a-chip can provide a system with higher throughput capable of mimicking the dynamic physicochemical environment hepatocytes and other liver cells encounter in vivo[162–164].

Co-differentiation. Co-differentiation considers deriving multilineage cell complexes starting with a single stem cell[111]. Growth factors are added sequentially to mimic different stages of liver development and stimulate multilineage cell specification. However, co-differentiation generates high cell type diversity at the expense of tissue architecture[38,39,165,166]. Hepatic, biliary and pancreatic bud organoids are the first three-organoid systems in which liver, pancreas and biliary duct tissue develop together[167]. This approach could provide a more physiologically relevant model of organogenesis as the development of the liver and pancreas occurs in a coordinated fashion. Endodermal hepatic organoids (eHEPOs) were generated from iPSCs using EPCAM-positive endodermal cells as an intermediate in 2 weeks and were expanded long-term (>16 months)[168]. They can model citrullinaemia type 1, a urea cycle disorder caused by mutations in the *ASS1* gene. The disease-related ammonia accumulation phenotype in eHEPOs could be reversed by overexpressing the wild-type *ASS1* gene, indicating that this model is amenable to genetic manipulation. Thus, eHEPOs provide an unlimited source of cells to generate functional liver organoids in a fast and efficient manner[168]. In 2024 Tadokoro et al. reported the development of hiPSC-derived liver organoids that mimicked the fetal liver at mid-gestation by fusing hundreds of hiPSC-derived liver bud organoids to induce static cell–cell interactions. HiPSC-derived liver organoids were successfully employed to reverse chemically induced liver fibrosis through transplantation[169]. Similarly, a study by Marsee and colleagues demonstrated that hepatobiliary organoids can be derived from hepatocyte spheroids formed by mature adult human hepatocytes, upon embedding in the ECM[170].

Assembloids. Assembloids represent more complex in vitro tissue- or organ-like structures formed from smaller, functional units, such as cells and tissues[171]. The term was derived from 'cell assembly'; it is a concept that was introduced by neuroscientist Donald Hebb in 1949, and refers to a group of interconnected neurons that are activated together in response to specific stimuli, making the group act as a functional unit[172]. Andersen and colleagues applied an adaptation of this concept to organoids produced by combining cortical organoids, spinal organoids and skeletal muscle organoids[173,174]. Although these do not fully fulfil the definition of assembly according to Hebb's original description, due to a lack of self-organization and the emergence of a single unit structure from the different parts, they indicate that several units can be put together to form a more complex structure.

The first attempt to generate more complex mouse liver in vitro systems came from Cordero-Espinoza and colleagues where they used the co-culture approach to increase cellular complexity. By co-culturing mouse ductal cells with mouse liver portal mesenchyme, they obtained ductal cell–portal mesenchyme organoids that retained the binary cell–cell interactions that occur in mouse liver[175,176]. This model was used to understand the role of portal mesenchyme during duct injury and regeneration, in which it controls ductal cell proliferation, at least in part, through cell–cell contacts[175]. Dowbaj and colleagues expanded this system by combining it with improved mouse hepatocyte organoids to generate mouse multicellular, complex structures that were named periportal assembloids. Periportal assembloids recapitulated the three-dimensional tissue architecture and cellular interactions of the periportal region of the mouse liver lobule[84]. In this system, near the portal mesenchyme, hepatocyte organoids created an interface with bile duct cells, enabling the functional connection between canaliculi and ductular lumen. Not only did this coculture model show improved cellular complexity, but an increase in the number of portal mesenchymal cells in the assembloid system resulted in the acquisition of a biliary fibrotic state[84]. Potentially, this system could be used to explain the persistence of the fibrotic state in the tissue, and these findings could open doors for understanding the persistence of the fibrotic state that eventually leads to liver cirrhosis and cholangiocarcinoma. It was shown in the same group that, by adapting the periportal assembloid system to human cells, combining human hepatocytes and cholangiocytes with portal mesenchyme from the same donor allows hepatocytes to acquire a portalized gene expression and functional programme[86].

An example of a pancreas assembloid is hESC-derived vascularized macrophage–islet organoids that consist of individually hESC-derived islet cells, macrophages and endothelial cells, which are subsequently aggregated to form functional assembloids unit. Using this system, pro-inflammatory macrophages were shown to

induce β-cell pyroptosis, at least partly, through TNFSF12 signalling upon SARS-CoV-2 and coxsackievirus B4 infection[177].

Transitioning towards fully chemically-defined extracellular matrices

Organoid cultures require a three-dimensional environment in which to grow and proliferate; this is often achieved by providing a scaffold for the organoids to reside in, mimicking the ECM in vivo. The ECM is a dynamic structure that provides mechanical, biophysical and signalling cues to facilitate the self-organization of organoids through the effects of cell shape, adhesion and differentiation. The most common ECM utilized in organoid systems is derived from Engelbreth–Holm–Swarm tumour in mice. It produces a large quantity of basement membrane components and has been commercialized as Matrigel[178]. Matrigel is composed of a multitude of structural proteins, growth factors, cell-binding proteins and undefined proteins, which help support organoid growth but also impose limitations on experimental systems[178]. In this section, we highlight the limitations of using animal-derived, potentially immunogenic ECMs such as Matrigel and Cultrex basement membrane extract.

Alternative approaches to using Matrigel to provide a three-dimensional scaffold include decellularized tissue hydrogels[179], natural polymer-based hydrogels[179] and fully chemically-defined synthetic hydrogels[63]. A strong driver for developing fully chemically-defined hydrogels is to achieve good manufacturing practice (a legal standard for the production of safe pharmaceutical products, commonly referred to as GMP). Developing compliant cultures will better enable clinical applications of organoid technology in which the physical, chemical and structural components of the synthetic hydrogel can be controlled and cell behaviour modulated[179,180]. The physical properties of synthetic hydrogels will be primarily determined by the polymer backbone (for example, polyethylene glycol, polyvinyl and dextran), which have been largely used in tissue engineering and regenerative medicine, and cell adhesion motifs (for example, the arginylglycylaspartic acid (RGD) motif is commonly used due to its interaction with integrin ligands[63,181]). To date, the composition of synthetic hydrogels capable of supporting organoid cultures has mainly been tissue-specific.

The generation of human ductal pancreas organoids was shown in a dextran–RGD-based synthetic hydrogel, which retained the expression patterns of organoids generated in Matrigel[63]. This synthetic hydrogel, however, supported a limited number of passages. To progress this technology to clinical applications, the issue of the limited number of passages will have to be addressed to enable the production of greater numbers of cells. Ductal liver organoids have been demonstrated in polyethylene glycol–RGD hydrogels[182], the properties of which could be modulated to recapitulate aberrant liver stiffness associated with fibrosis, opening up new avenues in disease modelling.

Utilizing a chemically-defined hydrogel provides opportunities to modulate signalling and ligand density, and mimic physiological stiffness and other biophysical properties of the hydrogel. Understanding how the composition of a synthetic hydrogel affects mechanical and cell signalling might facilitate the production of a single, defined hydrogel to support multiple organoid systems and simplify workflows. Modulating synthetic hydrogels might also be key to enabling the coculture of multiple cell types. Beyond organoid culture, the tunability of these hydrogels will also be an important factor in developing other artificial liver models using liver-on-a-chip technologies and three-dimensional bioprinting.

Conclusions

Development of new in vitro systems has ramped up in the past decade, especially with the establishment of new fields (for example, organoids) and the development of new or the application of known techniques on cell biology (for example, microfluidics and bioprinting)[183]. As happens with developing fields, it requires time to determine suitable pipelines to develop, characterize and benchmark systems so that the broader scientific community can readily use them. Very often, new models are reported, but without a clear idea of application, regardless of the significance of the improvement compared with older models. This 'paradox of choice' makes it difficult for the scientific community to choose which system to use for tackling new scientific questions, resulting in the generation of new models. One needs to balance the pragmatic (for example, cost and time to make the culture) and scientific (that is, is this model suitable to address the hypothesis?) reasoning when deciding on a model to use. And when none of the described models fits the purpose, the investment in developing a new one is justified.

Liver and pancreas organoids have become invaluable tools for modelling tissue biology, understanding disease mechanisms, and exploring therapeutic applications. From the pioneering work on mouse and human liver ductal tissues to the generation of organoids from pluripotent cells harbouring multiple structures, these models provide a versatile platform for basic and translational research. Advances in liver and pancreas organoid technology are yet to enable detailed studies of liver development, disease pathogenesis, and the testing of therapeutic interventions. As research progresses, liver organoids are expected to play a crucial part in advancing our understanding of liver biology and improving the treatment of liver diseases.

However, we, as a scientific community, need to be mindful of the extent of recapitulation of in vivo tissue observations into the in vitro cultures we develop. The ideal scenario is to reproduce in vitro, as closely as possible, the in vivo tissue. Nonetheless, deviations from this 'ground-truth' homeostatic tissue architecture have been shown to be useful in understanding the pathology of the organs. A prime example is apical bulkheads, F-actin-rich transverse structures connecting opposite sides of the bile canaliculus, which were first observed in mildly cholestatic two-dimensional hepatoblast cultures[184]. In a follow-up study, their appearance in vivo was correlated with cholestatic liver diseases, where their presence in human tissue was proposed to be used even as a hallmark of early stages of liver diseases such as primary sclerosing cholangitis[117]. Hence, this observation in two-dimensional-cultured hepatoblasts enabled the investigation of the subcellular dynamics in the development of cholestatic liver disease and indicated that the first hepatocyte organoid models were cholestatic in nature.

Finally, in line with George Box's quote from 1976, "All models are wrong, but some are useful"[185,186], we can also ask ourselves, when do we stop developing new systems? Not every scientific question requires organoids with multiple[153] cell types, nor does it require obtaining a full lobe. In fact, for a system to be useful, it also has to be easily accessible and highly implemented within the scientific community. Wide acceptance of highly complex models as routine models for studying physiology or pathophysiology is difficult. So, although we should aspire to achieving full mini-organs in a dish, completely vascularized and formed by all cells in the tissue, at each step, we can pause and study what valuable insights we can already obtain and how these can guide decision-making effectively. We do not need to have an organoid with multiple cell types to understand cell-autonomous

processes. However, to model the progression of a disease, we might need in vitro systems that encounter known players in this process. Finding the delicate balance between novelty and utility will be the next challenge.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

M.H. is inventor in several patents related to organoid technology. M.H and A.S. are inventors in a patent on assembloids. The other authors declare no competing interests.

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