

Organoids as an *in vitro* model of human development and disease

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The *in vitro* organoid model is a major technological breakthrough that has already been established as an essential tool in many basic biology and clinical applications. This near-physiological 3D model facilitates an accurate study of a range of *in vivo* biological processes including tissue renewal, stem cell/niche functions and tissue responses to drugs, mutation or damage. In this Review, we discuss the current achievements, challenges and potential applications of this technique.

The successful exploitation of human stem cells for clinical use has long been hampered by our inability to maintain and expand adult stem cells while retaining their multi-lineage potential *in vitro*. However, advances in our understanding of stem cell niches and the role of key signalling modulators in controlling stem cell maintenance and differentiation have fuelled the development of new 3D *in vitro* culture technologies that sustain stem-cell-driven formation of near-physiological, self-renewing tissues called organoids. The term 'organoid' has historically been used loosely to encompass all 3D organotypic cultures derived from primary tissues (either tissue subunits or single cells), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), established cell lines, as well as whole or segmented organs such as organ explants consisting of multiple tissue types¹. Here we define an organoid as an *in vitro* 3D cellular cluster derived exclusively from primary tissue, ESCs or iPSCs, capable of self-renewal and self-organization, and exhibiting similar organ functionality as the tissue of origin. Most of the documented organoid cultures contain functional tissue units that lack the mesenchymal, stromal, immune and neural cells that intersperse the tissue *in vivo*. These organoids rely on artificial extracellular matrices (ECM) to facilitate their self-organization into structures that resemble native tissue architecture.

The widespread implementation of organoid-based technologies across academia and industry is testament to their importance as near-physiological models for use in both basic and translational research. Unlike more traditional *in vitro* cultures, organoids are similar to primary tissue in both their composition and architecture, harbouring small populations of genomically stable, self-renewing stem cells that give rise to fully differentiated progeny comprising all major cell lineages at frequencies similar to those in living tissue. Another advantage is that organoids can be expanded indefinitely, cryopreserved as biobanks and easily manipulated using techniques similar to those established for traditional 2D monolayer culture. Finally, the fact that primary-tissue-derived organoids

lack mesenchyme/stroma provides a reductionist approach for studying the tissue type of interest without confounding influences from the local microenvironment. Organoids represent an important bridge between traditional 2D cultures and *in vivo* mouse/human models, as they are more physiologically relevant than monolayer culture models and are far more amenable to manipulation of niche components, signalling pathways and genome editing than *in vivo* models (Table 1).

In this Review, we discuss recent developments in murine and human organoid technologies from primary tissues as well as ESCs and iPSCs. We critically appraise the value of organoids as model systems for understanding human development and disease, as well as for clinical applications. We also highlight key challenges that remain to be addressed.

A brief history of organoids

Researchers have long known of the self-organizing capacity of mammalian cells and have harnessed this ability to generate 3D cultures from primary tissues, but the development of the intestinal organoid culture system in 2009 was a major technological advance for the stem cell field. Unlike previous systems, this new method made use of our knowledge of endogenous intestinal stem cell niche components to deliver a well-defined, stable culture system capable of sustaining the long-term growth of near-physiological epithelia from purified Lgr5⁺ stem cells or isolated crypts. The culture system was surprisingly simple, using Matrigel as an ECM substitute, supplemented with growth factors constituting key endogenous niche signals: WNT, a Frizzled/LRP (lipoprotein receptor-related protein) ligand; Noggin, a BMP (bone morphogenetic protein) inhibitor, to allow for stem cell expansion; R-spondin, an LGR4/5 (leucine-rich repeat-containing G-protein-coupled receptor 4/5) ligand, a WNT agonist to maintain stem cell populations; and EGF (epithelial growth factor), an EGFR ligand, to promote cell proliferation. As such, this culture system is commonly referred to as the R-spondin method. It was used to create 3D structures with distinct crypt-like and villus-like

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Table 1 Advantages, limitations and possible solutions to challenges faced with organoid cultures

Advantages	
Near-physiological model system for studying adult stem cells and tissues in a variety of contexts	
Adult stem cells can be propagated in organoids, and specific tissue lineages can be cultured in high purity with minimal contributions from other cell types (for example, fibroblasts and endothelial cells)	
Can be propagated for a long time (years) without genomic alterations	
Amenable to a wide variety of established experimental techniques	
Can be derived from multiple sources: adult and foetal tissues, ESCs and iPSCs	
Can generate organoids encompassing a broad range of tissues	
Limited amounts of starting material can be expanded for numerous applications	
Human diseases that are difficult to model in animals can be studied with patient-derived organoids	
Possibility of generating isogenic adult tissue for transplantation in regenerative procedures	
Limitations	Possible solution(s)
Lack of native microenvironment precludes studies about interaction of stem cells with their niches, immune cells, etc.	Complement with organotypic culture system, or co-culture with other cell types such as stromal cells ¹⁵ or immune cells
Limited use in modelling inflammatory responses to infection or drugs due to absence of immune cells in culture system	
Unable to mimic <i>in vivo</i> growth factor/signalling gradients in Matrigel matrix	Apply microfluidic technologies to generate concentration gradients
Unable to mimic biomechanical forces that stem cells encounter <i>in vivo</i>	Novel substrates and ECM factors are being identified to model such interactions <i>in vitro</i> ⁵⁰⁻⁵²
Relatively rigid ECM could limit drug penetration, hence hampering the use of organoids in drug screens	Devise ways to vary physical attributes of ECM such as composition, porosity and stiffness
Challenging to culture organoids from tissues whose niche factors are not well understood (for example, the ovary)	Screen for small-molecule modulators of key signalling pathways and specific hormones as potential culture components
Organoids in the same culture are heterogeneous in terms of viability, size and shape, impeding phenotype screens	Organoids can be tracked individually by live or time-lapse imaging
Organoid cultures depend on mouse-sarcoma-derived Matrigel, which precludes transplantation of organoids into humans	More defined ECMs that support organoid growth are being developed to comply with regulations for transplantation into humans

domains bordering a central lumen containing dead cells extruded from the constantly renewing epithelial layer² (Table 2 and Fig. 1). Remarkably, these organoids faithfully recapitulated the *in vivo* tissue architecture and contained the full complement of stem, progenitor and differentiated cell types. The system was subsequently adapted for generating human intestinal organoids, as well as organoids from other organs harbouring Lgr5⁺ stem cells, including the colon, stomach and liver³⁻⁷.

Organoids from primary tissue

Organoids have been successfully generated from many regions of the mouse gastrointestinal tract, ranging from the tongue through to the colon (Table 2). Spherical lingual organoids comprising a multilayer keratinized epithelium and stratum corneum were generated from isolated cells expressing Bmi1, an established marker of adult stem cells⁸. More recently, single cells expressing Lgr5 or Lgr6, two other well-known adult stem cell markers, successfully gave rise to functional taste bud organoids^{9,10}. Other studies have described the generation of ductal and lobular-type salivary gland organoids from salivary gland primary sphere cells plated in a mixture of Matrigel and collagen¹¹. In the oesophageal organoid cultures, the addition of Gastrin to the R-spondin-based culture media helped generate organoids that allowed researchers to identify cells involved in the self-renewal of oesophageal epithelium¹². Gastric organoids from the pylorus and corpus regions can also be grown using a variation of this R-spondin-based culture method. However, differentiation of the proliferative organoids requires withdrawal of WNT3A and FGF10 (fibroblast growth factor 10) from pyloric cultures, or WNT3A, FGF10 and Noggin from corpus cultures^{5,6}.

Establishing 3D organoids from other gastrointestinal epithelial organs has proven more challenging, requiring tissue-specific modifications that reflect the individual niche requirements and lineage commitment factors for the resident stem cell populations and their progeny (Fig. 1). The addition of various modulators of WNT (for example, CHIR99021, which inhibits GSK3 β (glycogen synthase kinase 3 β)) and Notch signalling pathways (for example, valproic acid, which inhibits histone deacetylase) has helped enrich and maintain the stem cell population, resulting in a more successful organoid culture¹³. A particularly exciting recent development has been the long-term growth of organoids from the adult liver and pancreas. Following acute injury, the pathways involved in bile duct and islet formation/regeneration were reactivated, facilitating the identification of the adult stem cell pool^{7,14}. Liver bile duct fragments plated into Matrigel and supplemented with EGF, R-spondin, HGF (hepatocyte growth factor), FGF10 and nicotinamide generated cystic organoids. These organoids contained cells expressing biliary ductal markers, which could be differentiated to a functional hepatocyte lineage by the inhibition of the Notch and TGF- β signalling pathways⁷. Adult tissue from pancreatic ducts was observed to generate budding cyst-like organoids when supplemented with EGF, R-spondin, FGF10 and nicotinamide. Isolated acinar cells could generate smaller, short-lived duct-like organoids, whereas cultures derived from endocrine cells were viable for 30 days but were non-proliferative. Importantly, engraftment of these pancreatic organoids under the kidney capsule resulted in the formation of functional pancreatic tissue containing ductal, endocrine and acinar cells, providing strong evidence that pancreatic stem cell potential resides in the adult ductal compartment¹⁴. In addition to adult

Table 2 Organoid models from various organs and ESCs/iPSCs

Tissue	Source	Organoid morphology	Cell types in organoid	Downstream applications tested	Types of human diseases modelled	Refs
Lingual	Mouse lingual stem cells	Spherical and budding organoids	-Stratum corneum -Keratin 5/14 ⁺ stem/progenitor cells -Granular cells of intermediate epithelial origin	-Tamoxifen inductions -Lineage tracing -Engraftment studies	Lingual carcinoma	8
Taste bud	Murine circumvallate tissue	Spherical and budding organoids	-Lgr5 ⁺ stem cells -Lgr6 ⁺ stem/progenitor cells -UEA/K8 ⁺ intragemmal taste bud cells -Gustducin-expressing, T1R3-expressing mature taste receptor cells -Sox9 ⁺ proliferative cells in budding regions	-Functional assays -Gene expression analysis -Cell cycle analysis	-	9,10
Salivary gland	Primary mouse cells	-Ductal branching organoids -Lobular spherical organoids	-CD24 ^{hi} /CD29 ^{hi} stem cells -Cytokeratin 7/18 ⁺ duct cells. -Aquaporin5-expressing acinar cells	-Gene expression analysis -Transplantation studies	Hyposalivation	11
Oesophagus	Mouse/human adult oesophageal tissue	-Spherical multilayered organoids -Human Barrett's oesophagus organoids: budding morphology	-CK14 ⁺ , p63 ⁺ small basal cells in the outer layer, large basal cells in the middle layer, and CK13 ⁺ cells in the central keratinized layer -Integrin α_4/β_4^{low} differentiated and integrin α_4/β_4^{hi} stem cells -Barrett's oesophageal organoids express goblet cells following differentiation.	-Tamoxifen inductions -Gene expression analysis	Barrett's oesophagus	3,12
Stomach	-Mouse/human adult tissue -Mouse/human ESCs -Human iPSCs	-Pylorus and corpus: spherical organoids -FGF10-driven budding events in pyloric organoids	-Pylorus: Lgr5 ⁺ stem cells, mucous neck cells, pit cells, enteroendocrine cells -Corpus: chief cells, mucous neck cells, pit cells	-Microinjection and infection model for <i>H. pylori</i> -Transcriptome profiling -Co-culture with mesenchymal cells -Adeno/retroviral transfections	- <i>H. pylori</i> infection -Cancer	5,6,23, 24,32, 33,70, 80-82
Intestine	-Adult tissue -Mouse/human ESCs -Human iPSCs	-Normal tissue: branching organoids -Diseased tissue: cystic and other morphologies	-Crypt-like domain contains Lgr5 ⁺ stem cells and paneth cells -Villus-like domain harbours villin ⁺ cells -Enteroendocrine and goblet cells scattered throughout organoid	-Transplantation studies -CRISPR/Cas9 gene editing -Cancer mutations -Transcriptome profiling	-Cancer -Cystic fibrosis -Infection model for viral and bacterial infection	2-4,13, 17-21,30, 31,53-55, 57-59, 61-64,72, 76,77,79, 83,84
Colon	-Adult tissue -Mouse/human iPSCs	-Normal tissue: budding organoids -Diseased tissue: cystic morphologies	-Lgr5 ⁺ stem cells -Enteroendocrine cells -Goblet cells -Enterocytes	-Transplantation studies -Transcriptome profiling -Biobank	-IBD -Cancer	3,21,22, 56,60,65
Liver	-Mouse adult tissue -Human iPSCs	-Mouse organoids: spherical -Human organoids: cystic	-Lgr5 ⁺ stem cells and bile duct cells -Hepatocytes observed after inhibition of Notch & TGF- β pathways -Human organoids contain Lgr5 ⁺ stem cells, ductal cells and hepatocytes -Cholangiocyte organoids express differentiation markers such as apical sodium-dependent bile acid transporter, secretin receptor, cilia and CFTR.	-Transplantation studies -Transcriptome profiling -Adenoviral transduction -Whole-genome sequencing -CFTR functional assays	-Alagille syndrome -Cystic fibrosis	7,25, 34-36,85
Pancreas	Mouse/human adult tissue	-E10.5 tissue: branching organoids -Adult tissue: budding organoids	-Ductal organoids contain Lgr5 ⁺ stem cells -Organoids from E10.5 murine pancreas can be differentiated into exocrine and endocrine cells	-Engraftment studies -Lineage tracing -Adenoviral transduction -Transcriptome profiling	Cancer	14,26,66
Prostate	Mouse/human adult tissue	-Normal tissue: spherical -Diseased tissue: branching similar to morphology of cancerous organoids	-Cytokeratin 5/p63 ⁺ basal cells -Cytokeratin 8 ⁺ luminal cells. Lgr4/Lgr5 ⁺ cells	-Tamoxifen inductions -Lentiviral infections	Cancer	27,28,86
Lung	-Mouse foetal pulmonary cells -Human ESCs/iPSCs	Spherical organoids	-Uniform proximal Sox2 and distal Sox9 expression -Proximal-like domains included basal, ciliated and club cells that were surrounded by smooth-muscle actin	-Transplantation studies -Cancer mutations -RNA sequencing	Cystic fibrosis	15,16,37
Retina	Mouse ESCs	Spherical organoids	-Outer layer recapitulates retinal pigment -Invaginated layer resembles retinal optic cup containing photoreceptors, ganglion cells, bipolar cells and Muller glia	-	-	38
Inner ear	Mouse ESCs	Budding organoids	-Functional prosensory vesicles -Otic vesicles also generate functional inner-ear hair cells	-	-	41,87
Brain	-Mouse/human ESCs -Human iPSCs -Patient skin fibroblasts	-Early-stage spherical organoids -Later-stage budding organoids	-Early-stage organoids contain continuous neuroepithelia -Cerebral cortical regions contain outer radial glia and cerebral cortical neurons	-Transfection -Transcriptome profiling -Electrical excitation	-Autism -Microcephaly	39,40, 73,88
Kidney	Human iPSCs	-Early-stage spherical organoids -Differentiated stages have budding morphology	Differentiated organoids exhibit segmentation as ducts, tubules and glomeruli	Toxicity screening in response to cisplatin	-	42

A summary of the organs that have seen success using the organoid model, including descriptions of the typical morphologies and cell types that have been observed, and the various diseases along with the associated techniques that have been modelled in the organoids. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.

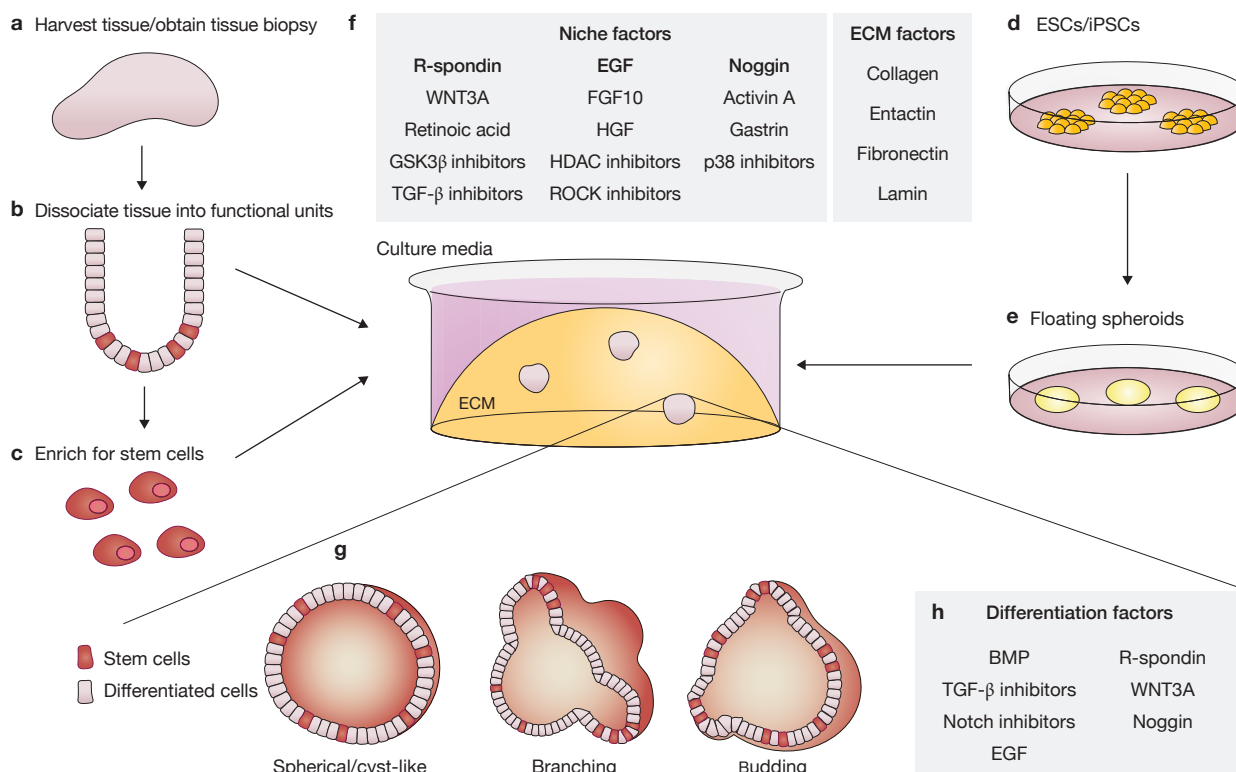


Figure 1 Organoid generation and culture from primary tissue and ESCs/iPSCs. (a–c) Organoids can be generated from primary tissue (a) that is dissociated into functional sub-tissue units containing stem cells (b). These are further digested into single cells and FACS-sorted to enrich for stem cells (c). Both the functional units and single stem cells can give rise to organoids under the appropriate culture conditions. (d,e) Organoids derived from ESCs and iPSCs (d) undergo directed differentiation towards the desired germ lineage, eventually generating floating spheroids (e) that are subsequently embedded in ECM to initiate organoid culture. Organoids are typically cultured in

extracellular matrix (ECM) surrounded by culture media supplemented with niche factors specific to the organoid type. (f) Common niche and ECM factors are listed, including factors constituting the ‘R-spondin method’ (see main text for further details). (g) Stem cells are maintained and perpetuated in organoids, continually giving rise to differentiated progeny. Typical morphologies are classified as spherical, branching or budding. (h) Organoids can either differentiate spontaneously or be induced to differentiate towards desired lineages or cell types by adding suitable differentiation factors (left column) and/or withdrawing factors that promote stemness (right column).

tissues, primary fetal tissue can also be used to generate organoids. Lung organoids were generated by manipulating VEGF (vascular endothelial growth factor), SHH (sonic hedgehog) and FGF signalling pathways in murine embryonic pulmonary cells that exhibited branching morphogenesis and sacculcation-like structures. As the process is remarkably similar to lung development *in vivo*, the pulmonary organoid culture provides a valuable *in vitro* tool for studying lung development^{15,16}.

Perhaps the most clinically relevant breakthrough has been the development of culture systems sustaining long-term growth and expansion of human organoids *in vitro*. In 2011, the first human organoids derived from both healthy and diseased oesophageal, intestinal and colonic epithelia were grown using the mouse R-spondin culture system supplemented with Gastrin, FGF10, TGF-β, MAP kinase inhibitors and nicotinamide, with other groups reporting similar success^{3,4,17–22}. It is now possible to culture organoids from human gastric tissue by varying concentrations of Gastrin and adding inhibitors of the TGF-β pathway to promote stem/progenitor cell maintenance^{23,24}. Human liver organoids have also been successfully derived from healthy biopsy samples. These cultures contained bi-potent stem cells that generated exclusively ductal epithelia under standard culture conditions, but retained the potential to produce mature hepatocytes when

differentiated in the presence of Notch inhibitors, FGF19, BMP7 and dexamethasone. Significantly, these organoids were capable of functional engraftment following transplantation into mice with acute liver damage²⁵. Employing a specific cocktail of WNT pathway activators and TGF-β pathway inhibitors, Boj *et al.* reported limited success in culturing human pancreatic organoids that could be maintained for up to six months and successfully cryopreserved²⁶. Propagation of mouse and human prostate epithelium as multi-layered spherical organoids harbouring bi-potent progenitor cells capable of differentiation towards both basal (outer layer) and luminal (inner layer) lineages has also been achieved using media supplemented with BMP receptor inhibitors²⁷. Such human prostate organoids comprising basal and luminal cell lineages can be generated from single normal human luminal and basal cells. Furthermore, tumour biopsies and circulating tumour cells from patients can generate organoids whose histologies mirror those of the primary tumours^{27,28}.

Organoids from ESCs and iPSCs

The use of murine and human ESC and iPSC lines to generate organoids circumvents the limited availability of high-quality human primary material, but requires detailed knowledge of the factors involved in

germ layer and subsequent lineage specification to perform directed differentiation. The use of iPSC lines requires an additional step compared to ESCs, as somatic cells first have to be converted into iPSCs through the expression of *OCT4*, *KLF4*, *SOX2* and *MYC*²⁹. Thereafter, ESCs and iPSCs are exposed to germ layer and tissue-specific patterning factors, followed by embedding in Matrigel to facilitate the development of 3D architecture, and treatment with differentiation factors to produce the intended organoids.

For endodermal tissues, TGF- β signalling is stimulated in ESCs and iPSCs to form definitive endoderm, which then differentiates into the relevant segment of the embryonic gut based on culture conditions. To generate intestinal organoids, FGF4 and WNT3A were applied to definitive endoderm cells from human ESCs and iPSCs to promote hindgut and intestinal fate, whereas another study cultured definitive endoderm from murine and human ESCs in fibroblast-conditioned media and with exogenous WNT3A (Table 2 and Fig. 1)^{30,31}. Recently, Noguchi *et al.*³² used murine ESCs to generate stomach organoid cultures that recapitulate the features of mature gastric cells found both in the corpus and antrum. By inducing *Barx1* expression and concomitantly manipulating the sonic hedgehog (SHH) and WNT signalling pathways, the authors were able to differentiate the definitive endoderm into foregut, and eventually establish near-physiological gastric organoids capable of secreting pepsinogen c and gastric acid, and displaying rudimentary peristaltic contractions. In contrast, a similar endeavour to generate gastric organoids from human ESCs and iPSCs by manipulating FGF, WNT, BMP, retinoic acid and EGF signalling pathways only reproduced cell types from the gland, pit and neck regions of the antral stomach, but lacked corpus cell lineages³³. Limited success was also reported for the liver, when iPSC-derived liver buds that also contained human endothelial and mesenchymal cells were cultured short-term before transplantation to generate vascularized, functional liver tissue³⁴. Recently, human iPSC-derived functional cholangiocyte organoids have also been generated by the modulation of the Activin A and Notch signalling pathways^{35,36}. For the lung, human ESC-derived endoderm was supplemented with Hedgehog pathway agonist to drive the sequential commitment towards foregut endoderm and finally spherical epithelial organoids expressing both proximal and distal lung markers observed during branching morphogenesis *in vivo*. However, no branching was observed in the organoid cultures, unlike those derived from primary fetal tissue^{15,16,37}.

In ectoderm-derived organoids, ESCs and iPSCs are induced to form embryoid body (EB)-like aggregates, which are then guided towards neural or non-neural fate after ectodermal specification. In the former, mouse ESCs have been used in combination with defined ECM components to generate region-specific retinal organoid cultures resembling the retinal pigment (outer shell) and embryonic optic cup (invaginated) structures³⁸. Additionally, two distinct but related methods have been developed to culture brain organoids from murine ESCs, human ESCs and iPSCs^{38–40}. The SFEBq (serum-free culture of embryoid body-like aggregates, quick) method pioneered by Eiraku *et al.*³⁸ generates organoids reminiscent of the telencephalon, whereas the cerebral organoid system of Lancaster *et al.*⁴⁰ employs a spinning bioreactor and produces organoids that recapitulate multiple regions of the brain. Nonetheless, brain organoids derived from both methods exhibited discrete cortical layers and proliferating progenitor zones that contained outer radial glial cells in patterns reminiscent of the early stages of human

brain development, and contained neurons that were fully functional and capable of electrical excitation^{38–40}. The brain organoid cultures are expected to be a valuable *in vitro* model for studying the intricate and complex processes occurring during human brain development. Furthermore, inner ear organoids from non-neural ectoderm harbouring prosensory cells and hair cells with mechanosensitive properties have been derived from murine ESCs by manipulating the BMP, FGF and TGF- β signalling pathways⁴¹.

Recently, the first successful mesoderm-derived organoids were reported: renal organoids were produced by modulating GSK3 β and FGF signalling pathways in human iPSCs, through an intermediate mesodermal state. These organoids recapitulate the morphology and segmentation of human fetal nephrons into ducts, tubules and glomeruli⁴². The human renal organoids provide a 3D model to study human renal development and disease under well-defined conditions, thus overcoming various limitations of previous models such as 2D monolayers, short-term 3D aggregates and co-cultures with mouse fibroblasts^{43–46}.

A notable difference between organoids derived from primary tissue and ESCs/iPSCs is the presence of cell types other than the intended lineage in the latter. This is because the factors used for directed differentiation of ESCs/iPSCs are not completely efficient in driving all the cells towards the lineage of choice, thus many ectodermal and endodermal organoids, such as those of the intestine, stomach and kidney, have reported the limited presence of mesenchymal cell types^{31,33,42}.

Despite these impressive technological advances, there are still tissues that remain resistant to organoid culture, but have been successfully cultured in 3D as whole-tissue explants or organotypic/mechanically supported cultures (for example, skin or ovary)^{1,47,48}. Central to the successful propagation of organoid cultures is the understanding of the endogenous stem cell niche and signalling pathways controlling lineage specification in these tissues. Thus, our relatively poor knowledge in these aspects for certain tissues precludes our ability to rationally design a complement of niche factors conducive to generating organoids. Although it can be argued that identifying the stem cells is not critical for culturing primary tissue units, the understanding of the stem cell niche will be crucial for the sustenance and indefinite propagation of cultures. A potential solution to this would be to screen for small-molecule modulators of key signalling pathways and organ-specific hormones as potential culture components supporting organoid growth from organs such as the ovary. Another potential complicating factor for organoid growth from certain tissues may be a strict dependence on growth factor/signalling gradients for maintaining balanced stem cell renewal and lineage specification. To circumvent this problem, microfluidic technologies could be used to create concentration gradients more comparable to the *in vivo* situation. Finally, it is evident that *in vivo* stem cell behaviour and cell differentiation are also heavily influenced by local biomechanical forces such as those resulting from interactions with the extracellular matrix, which are difficult to replicate *in vitro*⁴⁹. Efforts are underway to screen for substrates and ECM factors that regulate cell behaviour *in vitro* in the hope that this will lead to more robust organoid culture models for a wider range of tissues^{50–52} (Table 1).

Applications of organoid technology

The capability to grow near-physiological, self-renewing organoids in culture provides us with an excellent model system for a wide range of both basic research and translational applications. A major advantage

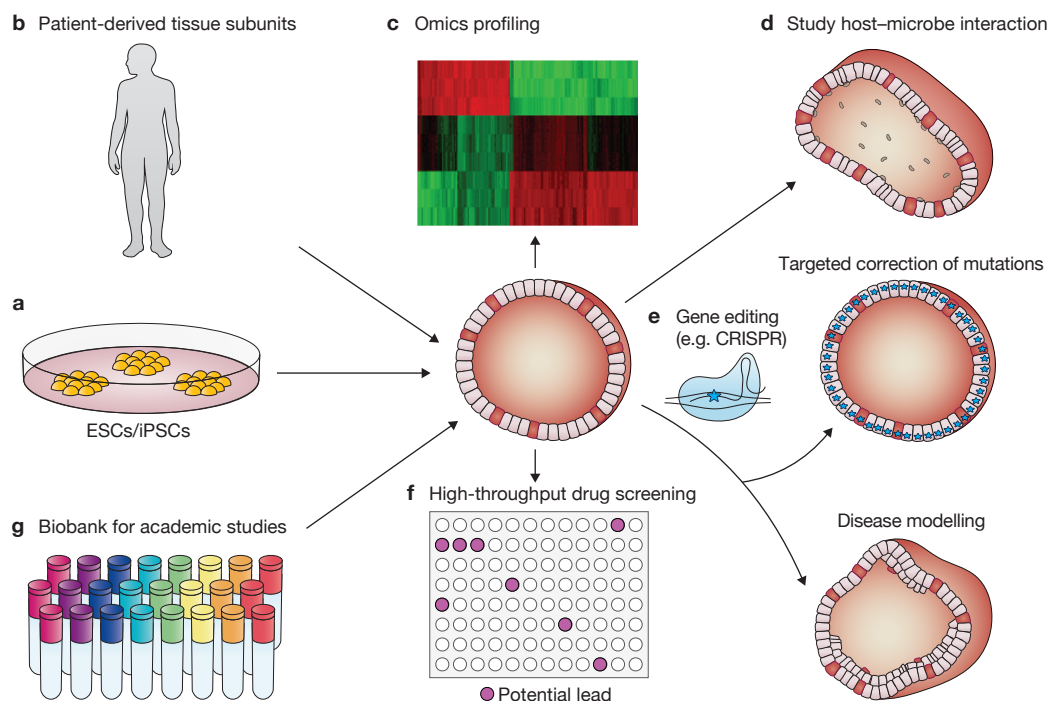


Figure 2 Applications of organoid technology for studying development, homeostasis and diseases. (a,b) Murine- and human-derived organoids from ESCs/iPSCs (a) as well as tissue subunits (b) contribute to the study of development and homeostasis. (c) Genome, transcriptome and proteome profiling of the organoids enables delineation of the contribution of various signalling pathways in development and their dysregulation in disease. (d) Bacterial and viral infections can be studied by injecting

infectious agents into the lumen of organoids. (e) Human disease can be modelled by biochemical and genetic manipulations to identify driver mutations and key signalling pathways. (f) Organoids represent a physiological model that can be used in high-throughput screens for effective drugs and small compounds at the preclinical stage. (g) Importantly, human organoids are being collated and catalogued as open-access biobanks.

of this system is the ability to greatly expand both tissue-specific stem cells and their differentiated progeny from very limited amounts of starting material such as biopsies, facilitating in-depth analyses of stem cell behaviour, drug screening, disease modelling and genetic screening. Indeed, the intestinal organoids have already been used extensively for analysing stem cell behaviour, identifying niche components, modelling pathogen–epithelia interactions, gene editing, disease modelling and orthotopic transplantation^{2,4,17–21,53–64} (Fig. 2). This success has spurred efforts to create cryopreserved biobanks of healthy and diseased human organoids as a renewable resource that is accessible to researchers worldwide⁶⁵ (Fig. 2g).

Organoids in the study of tissue development and disease. The organoid system allows researchers to intensively study the processes that govern embryonic development, lineage specification and tissue homeostasis, as well as the onset and manifestation of disease. As organoids generated from ESCs, iPSCs and fetal tissues faithfully retain the features of their original developmental stage (Fig. 2a), we can obtain detailed snapshots of embryonic development in a dish as differentiation of the cells is systematically induced. It also delivers invaluable mechanistic insight into the development of stem cells and their niches, while providing an opportunity to monitor their differentiation into mature functional lineages. As an example, fetal pulmonary organoids are being used to determine the signalling interplay between exogenous FGFs essential for endothelial network assembly and the VEGF-A pathway that inhibits the formation of the endothelial network, as well as the crosstalk with the

SHH pathway that induces epithelial and endothelial morphogenesis^{15,16}. Similarly, the development of tissues such as the stomach, brain and pancreas has been studied through stepwise differentiation of iPSCs and ESCs to organoids. This is achieved by modulating signalling pathways such as WNT, BMP and FGF, thereby elucidating the signalling network that ultimately patterns these tissues^{33,40,66,67}. The self-renewing capacity of organoids facilitates the expansion of primary epithelia from very limited starting material for expression profiling studies or for analysing rare cell lineages that are difficult to access *in vivo*⁶⁸ (Fig. 2c).

Another exciting application of the organoids is modelling host–microbe interactions (Fig. 2d). *Helicobacter pylori*, a causal agent of gastritis and gastric cancer onset in humans, was recently found to efficiently colonize the luminal epithelia of human gastric organoids, resulting in major physiological changes, including an increase in proliferation due to oncogenic CagA and increased β -catenin signalling^{23,24,69,70}. Additionally, some 3D tissue models have been applied to the study of microbial pathogenesis such as haemolytic uremic syndrome caused by Shiga-toxin-producing *Escherichia coli*⁷¹. Here, the renal organoids can provide a window to study the cells colonized by the bacterium and the subsequent manifestation in the tissue. As more organ systems become amenable to organoid cultures, the study of bacterial and viral infection and manifestation will allow a greater understanding of the pathogenic mechanisms and subsequently lead to better treatment strategies.

Other studies have employed CRISPR/Cas9-mediated gene editing of healthy organoids to directly evaluate candidate gene function in tissue physiology and carcinogenesis (Fig. 2e). This approach was used to

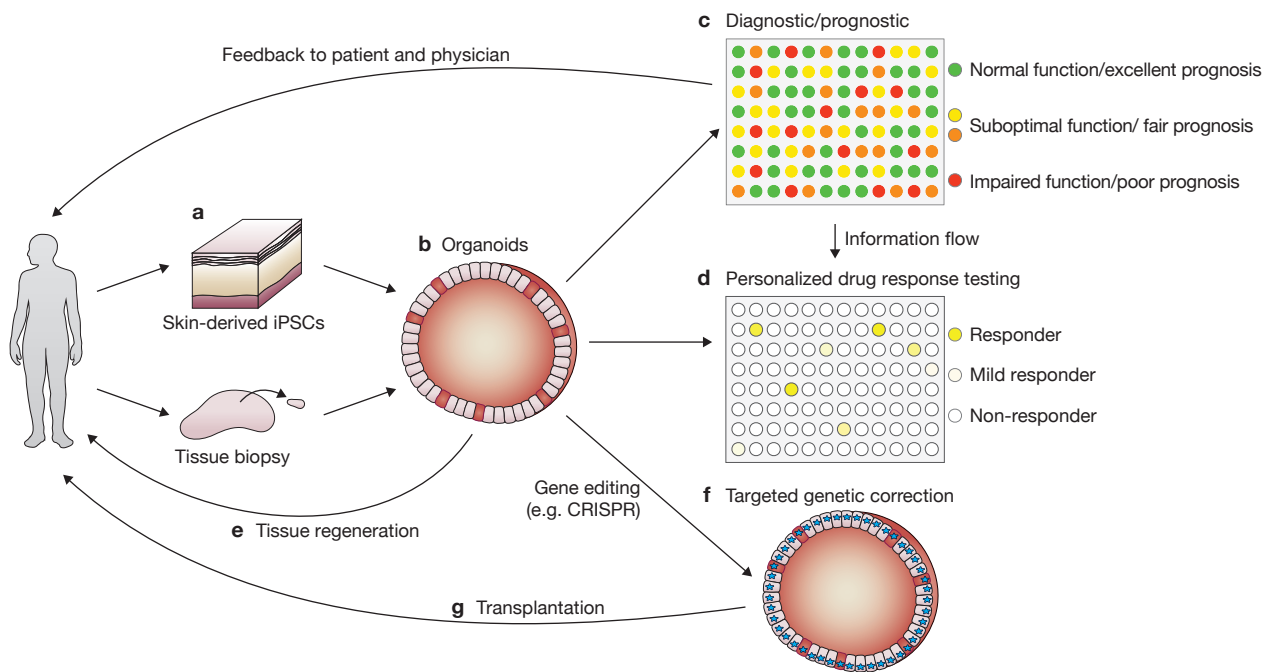


Figure 3 Potential therapeutic and diagnostic uses for organoid technology in personalized medicine. (a) Human iPSC- or tissue-derived organoids can contribute to the design of personalized treatment strategies. (b) The diseased and normal matched organoids can then be used to assess tissue function and prognosis in response to various treatments. (c,d) A wide variety of active drugs and small compounds can be screened for targeting candidate signalling

pathways (c) to design more effective drug regimens (d) in conjunction with other relevant diagnostic and prognostic factors. (e–g) The organoid cultures can also provide an expansive source of tissue for regeneration (e) and transplantation. Further genetic manipulation of organoids (f) can help restore functionality and normal physiology, and can be transplanted into donors for the re-establishment of physiological function (g).

introduce serial mutations into healthy human colon organoids, converting them into cancer organoids capable of driving *in vivo* cancer formation following orthotopic or kidney capsule transplantation^{17,21,72}. Murine organoids have also been used to model the role of oncogenic Kras in pancreatic neoplasia, accurately recapitulating the human disease. By comparing the gene expression and proteomic profiles of murine organoids with normal or oncogenic Kras, the authors identified signalling pathways and driver genes crucial to the progression of adenocarcinoma²⁶.

Certain human diseases have been extremely difficult to model in animals, such as those affecting human brain development. Therefore, patient-derived organoids from adult tissue and iPSCs present an alternative means to dissect the pathologies of these diseases (Fig. 2a,b). For example, studying neural organoids from patients with microcephaly due to loss-of-function mutations in the *CDK5RAP2* gene showed that non-functional *CDK5RAP2* led to premature neural differentiation, resulting in brain hypoplasia⁴⁰. Similarly, transcriptome profiling of organoids derived from iPSCs of autistic patients led to the discovery that these patients exhibit overproduction of GABAergic inhibitory neurons⁷³.

Potential of organoids in therapeutics and drug development. Many existing 2D cell lines harbour multiple culture-induced mutations or contamination with other cell lines that limit their value as accurate models for disease modelling or drug screening applications^{74,75}. Indeed, over-reliance on such inherently non-physiological models is likely to have contributed to the high failure rate of many drug discovery programs over the past two decades. This has fuelled efforts to develop high-throughput screening methods incorporating the far more stable,

physiological patient-derived organoids for use in early drug discovery programs and toxicity screens^{76,77}. Recently, Ogawa *et al.* were able to correct CFTR (cystic fibrosis transmembrane conductance regulator) misfolding and translocation to cell membranes in patient-derived cholangiocyte organoids, using inhibitors to reduce misfolding and stabilize the protein³⁶. This demonstrates the utility of the organoids for testing and screening novel compounds to treat various conditions (Fig. 2f).

Patient-derived organoids also represent an important resource for developing personalized treatment regimes (Fig. 3a,b). *In vitro* amplification of patient organoids from disease-site biopsies can deliver sufficient material for deep sequencing to reveal causal mutations, or for in-depth phenotypic profiling to facilitate more tailored treatment regimes (Fig. 3c). The ability to grow matched healthy and diseased organoids from human patients additionally enables clinical screens for drug combinations that selectively target the diseased tissue, helping to identify more effective treatments with minimal side effects (Fig. 3d). Many side effects of anti-cancer drugs can be attributed to acute liver toxicity. One could therefore envisage using hepatic organoids for predicting *in vivo* liver toxicity of experimental drug combinations before commencing expensive clinical trials⁷⁸. Other clinical applications include the use of disease organoids for predicting acquisition of drug resistance and for developing drugs that effectively target candidate cancer stem cells. Furthermore, in combination with 4D microscopy, organoids can be tracked over time to assess cancer stem cell behaviour and viability in response to active drugs to predict patient outcomes.

Although the development of organoid models represents a major technological breakthrough, the limited presence (if not complete lack) of stromal components, including immune cells, limits their use

in modelling inflammatory responses to infection or drugs. Another caveat for organoid use in drug-screening programs is potential limitations to drug penetration resulting from the relatively rigid ECM, which could in part be addressed by varying the physical attributes of the ECM (Table 1). Furthermore, variation of ECM physical attributes such as composition, porosity and stiffness should also facilitate modelling of interactions between ECM and the invasive front of tumours, furnishing a means of screening for novel drugs blocking tumour-mediated remodelling of ECM during tumour growth and invasion. Unfortunately, organoid cultures are often intrinsically heterogeneous in terms of viability, size and shape, which complicates the analysis of drug toxicity and efficacy. Development of live imaging techniques that facilitate real-time analysis of organoid response and detailed characterization of the heterogeneity within the culture would help to overcome this limitation (Table 1).

Organoids and regenerative medicine. Modern medicine is capable of replacing damaged and/or non-functional tissue with healthy tissue by allogenic transplantation, but a limited supply of healthy donor tissue and the inherent complication of tissue rejection highlight the need for additional tissue sources. Organoid technologies equip researchers with the ability to expand isogenic tissue from minuscule patient biopsies for transplantation use (Fig. 3e). Using iPSC technologies, it is also possible to generate a suite of isogenic or HLA-matched tissue-specific organoids from readily accessible tissue biopsies (for example, skin). Patient organoids harbouring genetic defects can now be repaired using gene-editing technologies to generate healthy isogenic epithelia for use in orthotopic transplantation as an effective treatment regime (Fig. 3f,g). The feasibility of such an approach was demonstrated by Dekkers *et al.*, who employed CRISPR/Cas9 gene editing with patient-derived colon organoids to correct germline CFTR mutations, thereby restoring enzymatic function to generate healthy epithelia capable of repopulating diseased tissue following transplantation⁴. Indeed, the capacity of *in vitro* cultured organoids to repair diseased or damaged tissue *in vivo* has been demonstrated by studies reporting functional engraftment of orthotopically transplanted organoids in the colon, pancreas and liver^{4,7,14,25,53,60,79}. It is expected that patient-derived organoids will be equally proficient in treating a range of human conditions, including ulcerative colitis, Crohn's disease and gastro-oesophageal reflux disease, as well as renal disease and hepatic cirrhosis. Although not currently possible, optimization of pancreatic organoid culture to maintain pancreatic progenitors and functional β -cells or islets of Langerhans could revolutionize diabetes treatment. Similarly, development of more physiological neural organoids would provide an excellent source of healthy tissue for treating a range of neurodegenerative diseases such as spinal cord injury and Parkinson's disease. Unfortunately, the reliance on mouse sarcoma-derived Matrigel as the artificial 3D matrix in organoid culture precludes the use of human organoids in clinical transplantation due to risks of unforeseen infection and immune/host rejection reactions. Hence, efforts are underway to design more defined ECMs that are compatible with clinical regulations for use in humans (Table 1).

Outlook and future prospects

Organoids are one of the most accessible and physiologically relevant models to study the dynamics of stem cells in a controlled environment

that can be derived from a variety of sources, as exemplified by the epithelial organoids. So far, they have proved to be a robust assay for establishing stem cell identities and niche compositions. In combination with genetic, transcriptome and proteomic profiling, both murine- and human-derived organoids have revealed crucial aspects of development, homeostasis and disease. The progress in generating organoids that faithfully recapitulate the human *in vivo* tissue composition has extended organoid applications from being just a basic research tool to a translational platform with a wide range of uses. The capacity to indefinitely culture organoids, without introducing genetic variation, makes them a sound model for high-throughput preclinical screenings, designing targeted and personalized therapies, and providing a source of fully functional tissue for regenerative medicine applications.

As interest in organoid technology grows, the commercial development of more standardized, validated organoid culture media will also be valuable in ensuring that the organoid system becomes accessible to a wide range of academic and clinical scientists, thereby helping to maximize its potential. Coupled with a more defined ECM, it is foreseeable that a highly accurate, reproducible culture model could emerge, overcoming current limitations that hinder the technology's transition from bench to bedside.

The organoid technology has synergized well with current methodologies and engendered a wide range of downstream functions and applications, underscoring its broad applicability and potential for manipulation. These features, in conjunction with the physiological relevance of the system, make organoids one of the most exciting and promising technologies that have emerged in recent times for studying human development, disease and therapy.

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ADDITIONAL INFORMATION

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The authors declare no competing financial interests.

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