Stem Cell-Derived Organoids in Disease

Subjects: Medicine, General & Internal

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Organoids represent one of the most important advancements in the field of stem cells during the past decade. The earliest usage of the term goes back to 1946 when Smith and Cochrane described a cystic teratoma case by referring to it as “cystic organoid teratoma”. Organoids, or as the term literally signifies “resembling an organ”, are three-dimensional (3D) in vitro culturing systems that originate from self-organizing stem cells, capable of mimicking the in vivo structural and functional specificities of an organ.

pluripotent stem cells embryonic stem cells organoids disease modeling 3D culturing

1. Introduction

Organoids represent one of the most important advancements in the field of stem cells during the past decade. The earliest usage of the term goes back to 1946 when Smith and Cochrane described a cystic teratoma case by referring to it as “cystic organoid teratoma” [1]. Organoids, or as the term literally signifies “resembling an organ”, are three-dimensional (3D) in vitro culturing systems that originate from self-organizing stem cells, capable of mimicking the in vivo structural and functional specificities of an organ [2]. They can be derived from either pluripotent stem cells (PSCs) or organ-specific adult stem cells (ASCs) [3]. PSCs, for instance, are capable of generating in vitro tissue models recapitulating what happens in in vivo organogenesis, where tissues are derived from embryonic stem cells (ESCs) [4]. **Figure 1** summarizes the potential sources for organoids development.

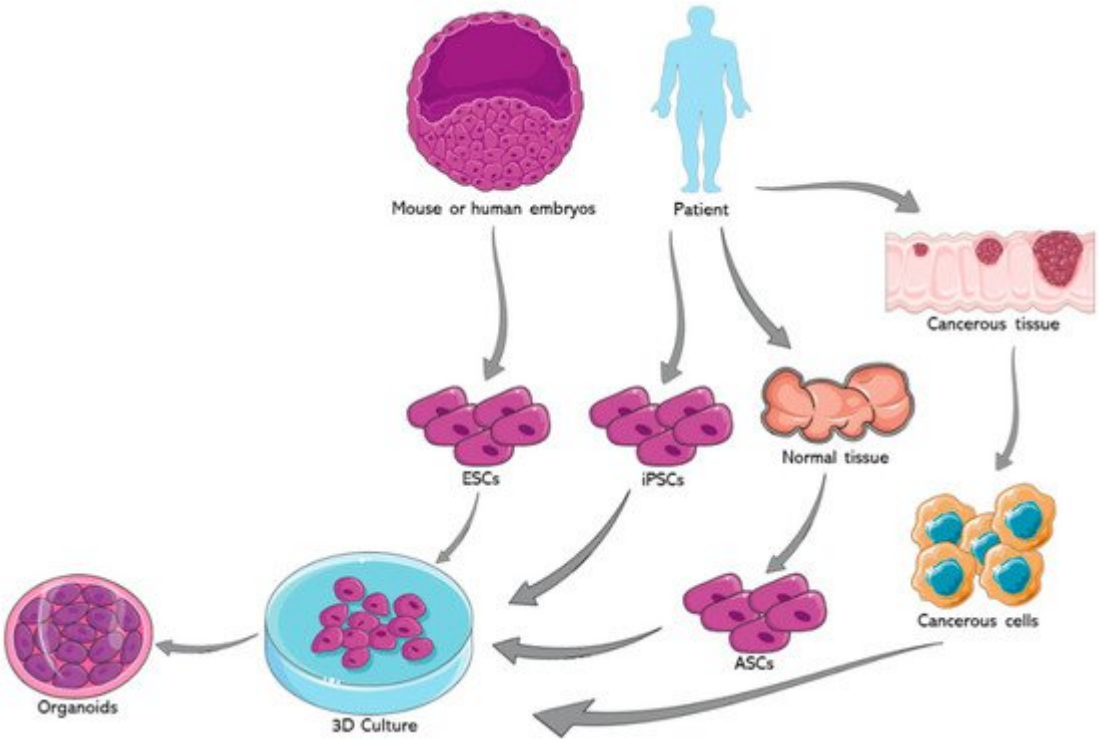

Figure 1. Organoids generation from different types of stem cells. Organoids can be generated from induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs) and patient-derived adult stem cells from normal and cancerous tissues (ASCs). Cells are usually grown in three dimensional systems under specific conditions to stimulate the growth of organoids. Shapes and images are imported from Servier Medical Art by Servier (http://smart.servier.com/), accessed on 24 May 2021. Licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Organoids have a great potential to be used in a multitude of fields [5][6]. One such avenue is cancer research, particularly through the development of tumor organoids. For example, glioblastoma organoids were established with the hopes of creating a satisfactory in vitro model that reflects the in vivo hypoxic gradients and heterogeneity of this greatly heterogenous cancer. This glioblastoma model can be used for both diagnosis and therapeutic purposes [7]. Furthermore, our group succeeded in generating and characterizing patient-derived prostate organoids from fresh primary tissue specimens. We confirmed the validity of those organoids as a relevant in vitro model to assess personalized treatment responses wherein we observed a differential drug response between different patient samples, at the protein and gene levels [8]. Moreover, we were able to isolate novel patient-derived prostate epithelial cells from the generated organoids [8]. Organoid models were also used in cancer immunotherapy research. In fact, Jenkins et al. used patient and murine derived organoids to establish an ex vivo profiling of PD-1 blockade. This would significantly help in dissecting the tumor microenvironment, enhancing precision immune-oncologic therapies, and ultimately deciphering the mechanisms behind immune checkpoint blockade resistance [9]. Furthermore, Dijkstra et al. co-cultured epithelial tumor organoids with peripheral blood lymphocytes to expand the population of tumor-reactive T-cells. This co-culture model can be also employed to characterize the sensitivity of neoplastic cells to the T cell-mediated killing at a personalized patient level [10]. Another potential avenue for the usage of organoids is hereditary diseases such as cystic fibrosis (CF), which

maintain the tissue of origin's genetic fingerprint. Beekman's group was able to elucidate a quantifying assay of the CF transmembrane conductance regulator (CFTR) function, the malfunctioning chloride channel in CF, using an intestinal model that closely mimics the core aspects of the disease's in vivo characteristics. This can aid in diagnosis and potentially provides novel therapeutic approaches to enhance drug development strategies for the disease [11]. After the COVID-19 pandemic has swept the world, organoids are proving to be of increasing value as viral replication platforms that can help expand our understanding of this virus [12].

Even though it has significant potential, organoid culturing is still rife with many challenges. Despite these systems' heterogeneity, the tumor microenvironment is often not properly reconstituted since most of the patient-derived organoids (PDOs) do not have the supporting stroma [13]. Other challenges observed with glioblastoma patient-derived xenotransplantation (PDX) models include cost, time consumption, and variable rates of transplantation [14]. Another limitation with the PDX system is that the immune response cannot be assessed properly since the host strains used are immunodeficient. Therefore, there is a poor assessment on how the immune system would have responded [15]. In this review, organoid culture will be thoroughly discussed along with its various applications in disease modeling (**Figure 2**).

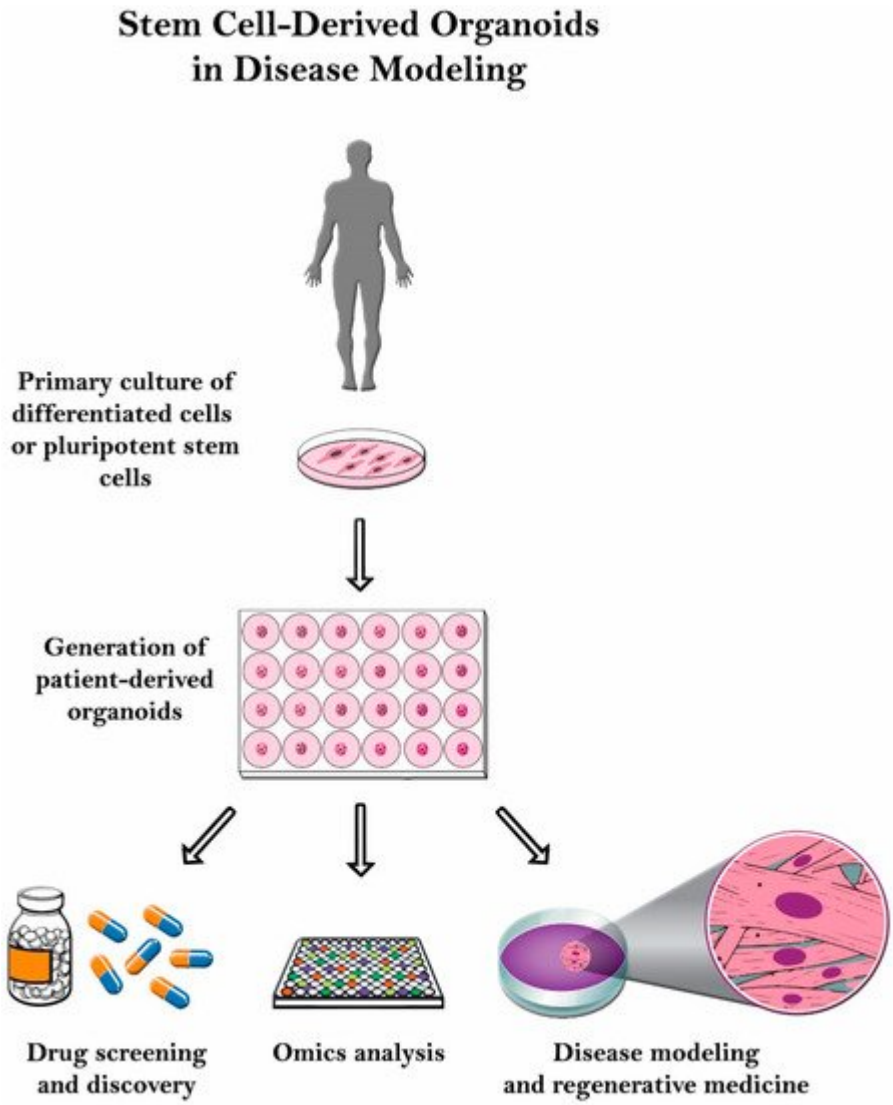

Figure 2. Schematic illustrating the use of stem cell-derived organoids for various biomedical and clinical applications including drug screening and discovery, disease modeling, genetic manipulation, omics analysis, and regenerative medicine among many others.

2. Modeling Diseases Using Induced Pluripotent Stem Cells (Ipscs)-Derived Organoids

Human iPSCs can be differentiated into functional thyroid tissue [16] which aids in cell-based regenerative therapy for hypothyroidism and in vivo production of circulating thyroid hormone [16].

As a final note, it is vital to mention that a considerable effort was dedicated to improving endoderm-derived organoid culture systems. For instance, Giobbe et al. suggested that extracellular matrix hydrogels derived from decellularized porcine small intestinal tissue can provide natural environment for the generation, stable growth, and in vivo transfer of endoderm-derived organoids [17]. The air liquid interface method that relies on collagen gels was recently employed to harbor the growth of 3D primary cells containing components of both mesenchymal and epithelial origin from mouse and human gastrointestinal tissues [18].

In a study performed by Huang et al., PSCs were successfully differentiated to pancreatic exocrine lineage mainly through TGF-β and notch inhibition to generate human pancreatic adenocarcinoma (PDAC) organoids [19]. iPSC-derived PDAC organoids were used to gain clinically important insights into PDAC as they maintained tumor-specific traits, inter-patient variation in tumor histoarchitecture, and showed differential responses to EZH2 inhibition as a therapeutic approach against PDAC [19].

Also, iPSCs isolated from patients with germline mutations that cause familial cancer predisposition syndromes modeled diseases such as Fanconi anemia [20], hereditary platelet deficiency with acute myeloid leukemia predisposition [21], and breast cancer predisposition [22]. Human iPSCs have also been used to model myeloid malignancies including juvenile myelomonocytic leukemia [23] and chronic myelomonocytic leukemia (CMML). Using CMML-iPSCs, Taoka et al. generated a humanized CMML mouse model and identified a MEK inhibitor, a Ras inhibitor, and liposomal clodronate as potential drugs for treating CMML [24].

3. Organoids from Embryonic Stem Cells (ESCs)

Recently, a 3D artificial thymic organoid model was reported to induce successful differentiation of hESCs to mature, functional, conventional T cells in vitro, with a varied T cell receptor repertoire. This method is relevant to the evaluation of human T cell development, and the establishment of likely adoptive T cell immunotherapies for cancer [25].

mESCs-derived embryoid bodies were employed to develop heart organoids containing cardiac muscle, conducting tissues, smooth muscle and endothelial cells that showed myocardial contraction and action potentials

[26]. Also, cardiomyocytes derived from hESCs cells proved to be useful in cardiac regeneration and in enhancing cardiac function in myocardial infarctions [27][28][29].

Recently, Lee et al. generated skin organoids, harboring epidermal and dermal layers, in vitro from mouse ESCs, under serum-free conditions. Furthermore, these skin organoids can spontaneously give rise to de novo hair follicles in a process that reproduces normal embryonic hair folliculogenesis. Hence, this in vitro 3D model of skin development will help study the mechanisms of hair follicle induction, assessing hair growth, testing drugs, and finally modeling skin diseases [30].

Organoids' properties also make them a powerful model to study the interaction between a host and infectious organisms such as Helicobacter Pylori or Salmonella Enteritica , gut-microbiota interactions, and inflammatory bowel disease, as well as the resulting inflammatory conditions. Furthermore, patient-specific responses to microbes can be mainly exploited [31]. The interaction between Clostridium difficile (C. difficile) and complex human epithelium was studied by using the HIOs. Viable C. difficile was introduced by microinjection techniques into the lumen of HIOs. Colonization of HIOs with C. difficile strain VPI 10463 leads to the disruption of the organoid epithelium. These effects seem to be caused by the primary virulence factors of C. difficile , the toxins TcdA and TcdB. This study shows that HIOs can be used for the comprehensive molecular and cellular analysis of the pathogenic interactions between C. difficile and human intestinal epithelium [32].

4. Organoids from Adult Stem Cells (ASCs)

Recent large cohort studies demonstrated that organoids derived from tumor tissues served as tools to predict patient response to chemoradiotherapy, whereby responses in patients matched patient-derived organoid responses [33][34][35][36].

ASC-derived organoid models have presented significant results within the modeling of human disease for a broad spectrum of life stages, early development and throughout to adulthood.

It is worth noting that there is a difference in ASC-derived organoid cultures between mouse and human systems. This has signified the need for human-based laboratory model systems to fully understand human development and pathophysiology.

Based on the source of cells, **Table 1** and **Table 2** highlight the differences in generation methods and recapitulation of the native organ's physiology respectively.

Table 1. Differences in generation methods of several organoid models based on the source of cells.

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
Intestinal	- Human-PSC-derived NCCs and developing human intestinal organoids were combined in vitro [43] - Patient-specific iPSCs harboring an LRRK2 G2019S mutation (LK2GS) [98] - Reprogramed fibroblasts from normal and FAP patients using the STEMCCA system [110] - A1ATD-1 hIPSCs derived from skin fibroblasts [122]	- Endoderm induction and differentiation into intestinal organoids [42]	- Co-culture of tumor cells and stromal cells supporting cancer development. Intestinal crypts and FAC sorted Lgr5+ intestinal epithelium cells embedded in Matrigel - Mouse- and human-derived colonic stem cells [230]
Lung	- hiPSCs [53,54,57]	- Endoderm induction and differentiation of hESCs to lung progenitor cells, 3D lung organoid formation, branching, and maturation [164]	- Lung epithelial cells sorted from human bronchi (excess unaffected areas). These cells had self-renewal and could generate luminal daughters [249] - Five subtypes (adenocarcinoma, squamous cell carcinoma, small cell carcinoma, adenosquamous carcinoma, and large cell carcinoma) of cancer tissues

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
			to generate human lung cancer organoids (LCOs) [273]
Cerebral	- Human iPSCs-derived embryoid bodies generated neuroectoderm which gave rise to cerebral tissues [89] - Miller-Dieker Syndrome (MDS) normal and patient-derived hiPSCs [94]	- Embryoid bodies formation, neural induction [175,176]	- Patient-derived glioblastoma organoids [263] - Patient-derived organoids from metastatic neuroblasoma patients [264]
Liver	- hiPSCs [47,48,49]	- Human expandable hepatic organoids (hEHOs) from hESCs with totally defined (serum-free, feeder free) media. 3D co-cultures of hepatic organoids were generated with hEHOs and human fetal liver mesenchymal cells to recapitulate the aspects of the human alcoholic liver disease-associated pathophysiology after ethanol treatment [171].	- Tumor organoids from patients who had primary liver cancer [268]

Table 2. Differences in the recapitulation of the native organ's physiology based on the source of cells.

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-derived Organoids
Intestinal	- Enteric nervous system development, neuroglial structures, functional interstitial cells of Cajal [43]	- Polarized, columnar epithelium that was patterned into villus-like structures and crypt-like proliferative	- Exhibition of an adult phenotype and recapitulation of the in vivo tissue architecture [230].

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-derived Organoids
	- Spheroid structures with a central empty lumen and some crypt-like structures comprised all intestinal epithelial cell types [98] - Gut organoids exhibiting definitive endoderm and intestinal specification. Differences in the generated intestinal-like tissues from normal or heterozygous APC iPSC were linked to the inter-patient variability [110] - Crypt-like structures, polarized epithelial cells, microvilli on the apical surface, enterocyte interspersed with Paneth, goblet, and enteroendocrine cells [122]	zones that expressed intestinal stem cell markers. The epithelium contained functional enterocytes, as well as goblet, Paneth, and enteroendocrine cells [42]	
Lung	- Mesenchymal and lung epithelial cells along with upper airway-like structures with basal cells, ciliated cells, and club cells surrounded by smooth muscle and myofibroblasts in addition to a distal-airway-like structures with bipotent alveolar progenitor cells [53] - Mesoderm and pulmonary endoderm, developed into branching airway and early alveolar structures and yielded growths containing tubular structures surrounded by mesenchymal tissue [54]	- Multiple cell types such as epithelial, mesenchymal, as well as ciliated cells. Organoids mimic the development and the function of a mature lung [164].	- Authors did not mention if they resembled the original epithelium, but they definitely do not resemble the organ's developmental physiology nor complexity because they lack other cells, such as stroma or columnar epithelial cells [249] - LCOs maintained the histology, and marker (CK7 and p63) expression pattern, and genetic characteristics (ex. Mutations in TP53 and EGFR) of the original tumor they were derived from [273]

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-derived Organoids
	- Synchronized modulation of proximal airway versus distal alveolar epithelial patterning [57]		
Cerebral	- Discrete brain regions including forebrain,- regional subspecification of cortical lobes and other regions such as the hippocampus, ventral forebrain, choroid plexus and immature retina [89] - Recapitulated MDS pathogenesis with horizontal cleavage planes in the ventricular zone, decreased vertical divisions, increased apoptosis of neuroepithelial stem cells, defective neuronal migration and abundance of CTIP2-positive neurons [94]	- Features of the human midbrain, but not the forebrain or the hindbrain [175] - Forebrain organoids which contain mainly dorsal forebrain neurons. Markers of midbrain and hindbrain were not observed [176]	- Organoids maintained biological features of high-grade glioblastoma (H&E staining), presence of the hall mark of glioblastoma, hypoxia and micro-vasculature (confocal imaging). Immunohistochemical analysis also showed the expression of neural progenitor and glioma stem cell markers. They also maintained molecular and intra- and inter-tumor heterogeneity [263] - The established organoids recapitulated the histological characteristics of the tumor (shown through H&E staining and immunohistochemical staining of NB84 (neuroblastoma diagnostic marker). The organoids also exhibited the disease-specific chromosomal aberrations, stemness properties, and tumor heterogeneity [264]
Liver	- Epithelialized cystic and/or ductal structures that express mature bile ducts markers including the CFTR marker, exhibit cystic	- hEHOs exhibit the phenotype of hepatic stem/progenitor cells. The cells from hEHOs	- Hepatocellular carcinoma tumoroids had compact structures, however, cholangiocarcinoma tumoroids

Organ Model	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-derived Organoids
	and/or ductal structures and epithelial functions including CFTR-mediated fluid secretion [47] - Cholanginocyte progenitors formed cystic organoids and branching tubular structures having primary cilia and expressing biliary markers similar to primary chlangiocyte, Cholangiocyte-like cells possessed functional activity including bile acids transfer, alkaline phosphatase activity, γ-glutamyltranspeptidase activity and physiological responses to secretin, somatostatin and vascular endothelial growth factor [48] - Vascularized and functional human liver. The generated liver tissue is capable of protein production and human-specific drug metabolism [49]	shows exceptional repopulation capacity in injured livers of FRG mice after transplantation, and they can differentiate in vivo into both mature hepatocytes and cholangiocytes [171]	had irregular cyst structure thus recapitulating native physiology [268]

5. Conclusions and Future Directions

In conclusion, although there might be many challenges hindering the use of organoids for clinical applications, this new technology holds great potential in translational research. Organoid technology has recently grown to incorporate drug screening, disease modeling, genetic manipulation, omics analysis, and many others (**Figure 2**). Experimental procedures that have been developed to culture organoids can be applied to different human organ systems, helping us better understand the human biology and development of diseases (**Figure 3**, **Table 3**). Indeed, human organoid systems are strongly proposed to offer exceptional opportunities to improve human health and move the biomedical and research fields to a whole new level of personalized patient-targeted medicine.

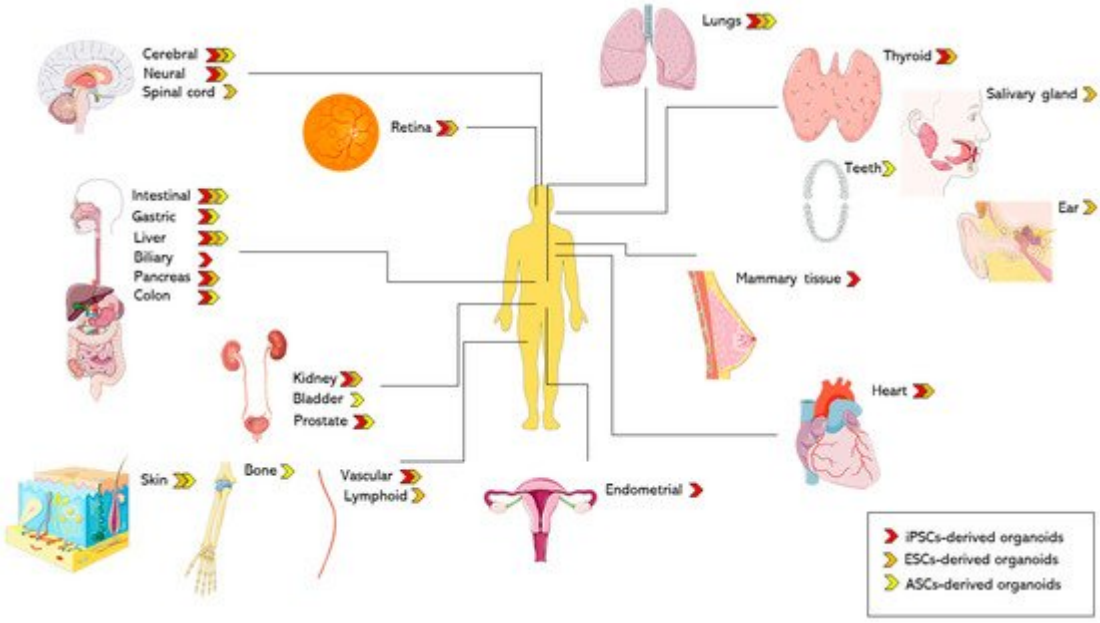

Figure 3. Reported organoid systems based on tissue of origin. Using different types of stem cells, many organs and tissues have been simulated using the organoids technology. Organ and human model images are imported from Servier Medical Art by Servier (http://smart.servier.com/), accessed on 24 May 2021. Licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Table 3. Table representing the multiple organ and disease models based on their respective organoid origin (iPSC, ESC and ASC).

	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
Organ models	▪ Intestinal [42,43,98,120,121,122,304] ▪ Gastric [41] ▪ Biliary [47] ▪ Liver [47,48,49] ▪ Thyroid [52] ▪ Lung [53,54,57,59] ▪ Kidney [62,63,67] ▪ Cardiac [68,69,70,72,73]	▪ Lung [164] ▪ Pancreas [165,166,167] ▪ Liver [171] ▪ Prostate [173] ▪ Midbrain [175] ▪ Brain cortex [176] ▪ Vascular [177] ▪ Lymphoid [178]	▪ Prostate [8] ▪ Intestinal [230,231] ▪ Colon [233] ▪ Stomach [232,233] ▪ Liver [234] ▪ Brain [39] ▪ Bone [243] ▪ Lungs [249,250] ▪ Teeth [253]

	iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
	▪ Vascular [85] ▪ Female reproductive tract [86] ▪ Endometrial [87,88] ▪ Cerebral [89,94,97,113] ▪ Neural [90,96,98] ▪ Ventral and dorsal forebrain [91,95] ▪ Neuromuscular junctions [99,100,101] ▪ Mammary tissue [102,103] ▪ Retina [104,105,106,107] ▪ Colon [110,111] ▪ Pancreas [112]	▪ Kidney [62,180] ▪ Thyroid [52,181] ▪ Heart [183] ▪ Ear [187,188] ▪ Salivary gland [190] ▪ Skin [191] ▪ Retina [195,209] ▪ Spinal cord [196,201] ▪ Motor neurons [197,198] ▪ Forebrain [199] ▪ Intestinal [212]	▪ Salivary gland [254]
Disease models	▪ Hirschsprung's disease [43] ▪ *Helicobacter pylori* infection [41] ▪ Alagille syndrome [48] ▪ Cystic fibrosis [47,48,59,238] ▪ Polycystic kidney disease [48,62,66] ▪ Barth syndrome [72] ▪ Genetic Cardiomyopathy [73] ▪	▪ SARS-CoV-2 infection [164] ▪ Diabetes [168,194] ▪ Steatohepatitis [171,172] ▪ Parkinson's disease [175] ▪ Polycystic kidney disease [62] ▪	▪ ZIKV infection and microcephaly [213,240,242] ▪ Gastric cancer [255,256,257] ▪ Colorectal cancer [258,259,260,261,262,284] ▪ Brain cancer [263,264] ▪ Pancreatic cancer [265,266,267,287] ▪ Liver cancer [268,285] ▪

iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
Cardiac Injury [68] ▪ Diabetic Vasculopathy [85] ▪ Endometrial disorders [88] ▪ Microcephaly [89] ▪ Autism spectrum disorder [90] ▪ Timothy syndrome [91] ▪ Tuberous sclerosis [93] ▪ Lissencephaly [94] ▪ Miller-Dieker syndrome [95] ▪ Alzheimer's disease [96] ▪ Huntington's disease [97] ▪ Parkinson's disease [98] ▪ Retinal ciliopathies [104] ▪ X-linked juvenile retinoschisis [105] ▪ Late-onset retinitis pigmentosa [106,107] ▪ Li-Fraumeni syndrome-associated osteosarcoma [108] ▪ Colorectal cancer [110,111] ▪ Pancreatic adenocarcinoma [112] ▪ Brain tumors [113]	Hypothyroidism [181] ▪ Myocardial infraction [184,185,186] ▪ Lesch-Nyhan syndrome [193] ▪ Retinoblastoma [209] ▪ Metastatic brain cancer [210] ▪ *Clostridium difficile* infection [212] ▪ ZIKV infection and microcephaly [213]	Bladder cancer [269] ▪ Prostate cancer [270] ▪ Breast cancer [271,286] ▪ Esophagus cancer [272] ▪ Lung cancer [273,274] ▪ Endometrial cancer [275] ▪ Cystic fibrosis [290,291] ▪ Rotavirus infection [293] ▪ Influenza virus infection [294,295,296] ▪ Enteroviruses infection [297] ▪ Human astrovirus infection [298] ▪ Human adeno-virus infection [299] ▪ Human papillomavirus infection [300] ▪ BK virus infection [301] ▪ Herpes simplex virus infection [300] ▪ Respiratory syncytial virus infection [274] ▪ SARS-Cov-2 infection [302,303,304,305,306] ▪ *Cryptosporidium* infection [307]

iPSC-Derived Organoids	ESC-Derived Organoids	ASC-Derived Organoids
▪ Middle East Respiratory Syndrome-related Coronavirus infection [119] ▪ Norovirus infection [120] ▪ Rotavirus infection [121] ▪ *Salmonella enterica serovar Typhimurium* infection [122] ▪ Herpes simplex virus 1 encephalitis [125] ▪ Respiratory syncytial virus infection [54]		▪ *Toxoplasma Gondii* infection [308,309] ▪ *Clostridium difficile* infection [212] ▪ *Salmonella enterica serovar Typhimurium* infection [309,310,313] ▪ *Listeria monocytogenes* infection [310] ▪ *Helicobacter pylori* infection [140,311,312] ▪ *Escherichia coli* infection [314,315,316] ▪ *Chlamydia trachomatis* infection [317] ▪ *Klebsiella pneumoniae* infection [318]

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