Stem Cell Research & Therapy

REVIEW

Open Access

From gut to liver: organoids as platforms for next-generation toxicology assessment vehicles for xenobiotics

Sulaiman Mohammed Alnasser[1*]

Abstract

Traditional toxicological assessment relied heavily on 2D cell cultures and animal models of study, which were inadequate for the precise prediction of human response to chemicals. Researchers have now shifted focus on organoids for toxicological assessment. Organoids are 3D structures produced from stem cells that mimic the shape and functionality of human organs and have a number of advantages compared to traditional models of study. They have the capacity to replicate the intricate cellular microenvironment and in vivo interactions. They offer a physiologically pertinent platform that is useful for the researchers to monitor cellular responses in a more realistic manner and evaluate drug toxicity. Additionally, organoids can be created from cells unique to a patient, allowing for individualized toxicological research and providing understanding of the inter-individual heterogeneity in drug responses. Recent developments in the use of gut and liver organoids for assessment of the xenobiotics (environmental toxins and drugs) is reviewed in this article. Gut organoids can reveal potential damage to the digestive system and how xenobiotics affect nutrient absorption and barrier function. Liver is the primary site of detoxification and metabolism of xenobiotics, usually routed from the gut. Hence, these are linked and crucial for evaluating chemical or pollutant induced organ toxicity, forecasting their metabolism and pharmacokinetics. When incorporated into the drug development process, organoid models have the potential to improve the accuracy and efficiency of drug safety assessments, leading to safer and more effective treatments. We also discuss the limitations of using organoid-based toxicological assays, and future prospects, including the need for standardized protocols for overcoming reproducibility issues.

Keywords Organoids, 3D cell culture, Toxicology, Drug toxicity, Developmental toxicity, Bioassays

Introduction

Organoids are multicellular, three-dimensional cultures made from stem cells. They assemble themselves into structures with cell types specific to each organ and imitate some of the in vivo cell structure and functions of the original organ [1, 2]. Organoids have a lot of potential for use in basic research and personalized therapy as our knowledge of organogenesis and tissue engineering develops [3]. Organoids also serve as disease models and facilitate drug screening through genetic alterations, exposure to disease-relevant stimuli, and toxicity and efficacy testing of medicinal substances, providing a closer-to-natural system than 2D cultures or animal models. This is because the traditional two-dimensional (2D) cell cultures failed to replicate the natural cell morphology and interactions found in vivo as they lose their normal shape, undergo aberrant splitting and flattening, and disrupt the differentiation phenotype over time [4]. This limits their relevance for studying human biology and disease mechanisms. Epidemiological studies also have their own limitations as they rely on observational

*Correspondence:
Sulaiman Mohammed Alnasser
sm.alnasser@qu.edu.sa
[1] Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Buraydah, Qassim, Saudi Arabia

data and can be influenced by confounding factors, such as lifestyle, genetics, and co-exposures [5, 6]. Establishing causal relationships between environmental toxicant exposure and health outcomes can be complex and may require large sample sizes and long-term follow-up [7, 8]. Additionally, epidemiological studies lack mechanistic insights into the toxicity of environmental pollutants. Systems toxicology approaches, which model the absorption, distribution, metabolism, and excretion (ADME) of chemicals, can also be limited in their predictive accuracy [9]. Similarly, animal models remain widely used in toxicology research but they have intrinsic drawbacks in predicting human responses to toxins due to species-specific differences in anatomy, physiology, and metabolism [10, 11]. These differences often lead to discrepancies in toxicity outcomes, making the extrapolation of animal data to human health risks challenging and potentially unreliable. Hence, 3D cultures or organoids are a promising alternative for overcoming these limitations [12]. By better recapitulating human tissue architecture and cellular interactions, they can significantly contribute to toxicological studies aimed at cellular interaction processes, assessment of the detrimental effects of toxins on cellular functions like adhesion, migration, differentiation etc. and drug response with improved accuracy [13–15].

However, while organoid models have made significant strides in replicating human tissues, they still fall short of fully replicating the complexity of native organs. One major limitation is the incomplete differentiation of certain cell types. For instance, in gut organoids, differentiation into all intestinal cell types, including Paneth (with a role in antimicrobial defense) and M cells (essential for immune surveillance), is often incomplete [16]. Additionally, the characteristic crypt-villus architecture of the intestine remains underdeveloped, limiting the ability of the organoid to fully model physiological processes such as nutrient absorption, immune interactions, and microbiome dynamics [17]. Similarly, liver organoids typically feature hepatocytes with fetal-like characteristics, lacking mature functions such as drug metabolism, bile secretion, and albumin production [18]. Both organoid models also struggle with insufficient vascularization and inadequate interaction with other crucial cell types, such as immune cells and fibroblasts, which are necessary for tissue maturation. Advancements in culture conditions, co-culture systems, genetic engineering, and microphysiological models are essential to improving the maturity and functionality of these organoids, ultimately making them more accurate and reliable models for disease research and drug testing.

Organoid technology is becoming instrumental in toxicology research as organoids provide a versatile platform for studying the toxicity of drugs, chemicals, and natural environmental toxins, offering human-relevant models that facilitate high throughput screening, mechanistic insights, and dose–response assessments [19–21]. As they can replicate the cellular makeup and structure of many organs, including the liver, kidney, brain, colon, and lung, they are being used to predict chemical exposures with greater precision by bridging the gap between traditional in vitro models and in vivo systems [20]. Researchers can apply specific toxin concentrations to organoids and observe how exposure affects cellular morphology, viability, and function [22]. This approach enables the identification of potentially harmful substances and provides insights into the fundamental mechanisms underlying toxicity at the cellular level. Organoids have been used to study the toxicity of environmental contaminants as well as the efficacy and impact of various drugs [23–25]. Pluripotent stem cell (PSC) derived organoids from patients offer the potential to test vast libraries of therapeutic compounds, bridging the gap between monolayer cell culture and animal models, enabling toxicity studies at a more physiologically relevant scale [26]. Processes such as cell migration, adhesion, differentiation, and apoptosis can now be studied in a context that closely mimics in vivo conditions. Recent innovations in co-culture systems allows researchers to include a broader range of cell types within the organoid models, such as immune cells or certain microbes alongside toxins, which can aid in recapitulating the full complexity of human tissue response to toxic substances.

Generation of organoids

Stem cell sourcing is the initial step in organoid creation, with options including adult stem cells (ASCs), induced pluripotent stem cells (iPSCs), or embryonic stem cells (ESCs) (Table 1). The choice of the source is based on specific organoid requirements [27]. ESCs are ideal for creating organoids that require multiple cell lineages due to their pluripotency, meaning they can differentiate into any cell type in the body [28]. However, ethical concerns surround ESC research due to the destruction of a blastocyst [29]. ASCs are multipotent and found in various adult tissues like bone marrow or fat [30]. They can differentiate into a limited number of cell types specific to their origin and this focused differentiation allows for more targeted organoid models. Hence, ASCs are readily available and avoid ethical issues, but their regenerative capacity is limited. iPSCs are reprogrammed from adult cells and share similar properties to ESCs, offering pluripotency and the potential to create any cell type [31]. They provide a solution to ethical concerns but may carry reprogramming-related risks. Depending on the reprogramming method, their availability and the consistency of cell quality can vary.

Table 1 Comparison of embryonic, adult, and iPSCs for generating organoids

Feature	ESCs	ASCs	iPSCs	References
Origin	Inner cell mass of a blastocyst (early embryo)	Various adult tissues (bone marrow, fat, etc.)	Reprogrammed adult cells	[32]
Pluripotency	Yes—Can differentiate into all three germ layers (endoderm, mesoderm, ectoderm) and become any cell type in the body	No (Multipotent)—Can differentiate into a limited number of cell types specific to their tissue of origin	Yes (Similar to embryonic)—Can potentially differentiate into all three germ layers	[33, 34]
Self-renewal	Yes—Can divide indefinitely while remaining undifferentiated	Yes (Limited)—Can divide for many cycles but eventually lose ability to self-renew	Yes—Can divide for extended periods	[35, 36]
Ethical Concerns	Yes—Destroys a blastocyst, raising ethical questions	No—No embryos harmed	Can vary—Depends on the method used for reprogramming	[29, 37]
Availability	Limited—Requires strict regulations and may not be readily available	More readily available—Can be isolated from various tissues	Potentially more readily available than embryonic	[38]
Immune Rejection	High risk—Cells are not genetically identical to the recipient	Lower risk—Can be obtained from the same patient (autologous)	Similar to embryonic (depends on reprogramming method)	[39, 40]

Several methods for generating organoids from stem cells or biopsies (in case of tumor derived organoids) have been developed to replicate essential aspects of organ function (Fig. 1). It typically takes a few weeks to produce mature organoids from iPSCs/ESCs and few days to produce from ASCs [34]. Stem cells or progenitors are seeded in 3D matrices, such as Matrigel or hydrogels, to provide a supportive microenvironment. Specific growth factors and small molecules are added to the culture medium to induce differentiation towards the desired cell types [41]. Cells self-organize into 3D structures that resemble the architecture of the target organ. However, the successful generation and maturation of organoids depend not only on biochemical cues but also on physical culture conditions, particularly agitation and oxygen availability [12]. Provision of nutrients and oxygen becomes increasingly challenging as organoid size increases. In static culture systems, organoids often face diffusion limitations, where nutrients and oxygen fail to penetrate efficiently to the core of larger structures [42]. This results in a gradient of nutrient availability, with cells at the periphery receiving sufficient sustenance while those in the center may experience hypoxia or nutrient deprivation. Consequently, organoids grown under static conditions typically exhibit a size limitation, rarely exceeding 100–200 µm in diameter [43, 44]. This constraint not only affects their growth but also limits their ability to fully model the complexity and functionality of native tissues. Agitation-based systems, such as bioreactors and orbital shakers, enhance organoid growth by improving nutrient and oxygen diffusion, ensuring uniform distribution, and reducing gradients [45]. This dynamic environment supports larger organoids while preserving structural integrity and preventing excessive aggregation. However, excessive mechanical stress can disrupt organoid organization, and careful calibration of agitation parameters is needed to balance growth enhancement with structural preservation [46]. Hence, further refinement of these systems is needed to fully harness their potential in organoid research. Different organoid models require tailored culture conditions to accurately replicate their in vivo counterparts since variations in tissue architecture, cellular composition, and functional requirements influence their growth and maturation. This review is focused on gut and liver organoids so an overview of their generation is provided in the subsequent text.

Gut organoids are derived from gut epithelial cell lineage (initially from mouse Lgr5 + adult stem cells and also known as enteroids), resembling the pyloric epithelium organized around a central lumen [47]. Human adult stem cell derived gastric organoids were later developed from various regions of the stomach, forming budding or cystic structures composed mainly of mucous gland, chief, and enteroendocrine cells surrounding a central lumen [1, 48]. Although gut organoids can grow in vitro without a mesenchymal niche, they rely on specific factors and extracellular components that mimic this environment. Key elements include Matrigel and a combination of biological enhancers such as Noggin, epidermal growth factor, R-spondin-1, and Wnt3a [49]. To better replicate physiological conditions, hypoxic environments (typically 5% O_2 or less) are often used to mimic the physiological oxygen levels in the gut [50]. To maintain growth and prevent spontaneous differentiation, organoids are mechanically or enzymatically dissociated and re-embedded in fresh Matrigel every 7–10 days [51].

Additionally, human gastric organoids can be further differentiated into various gastric cell lineages by

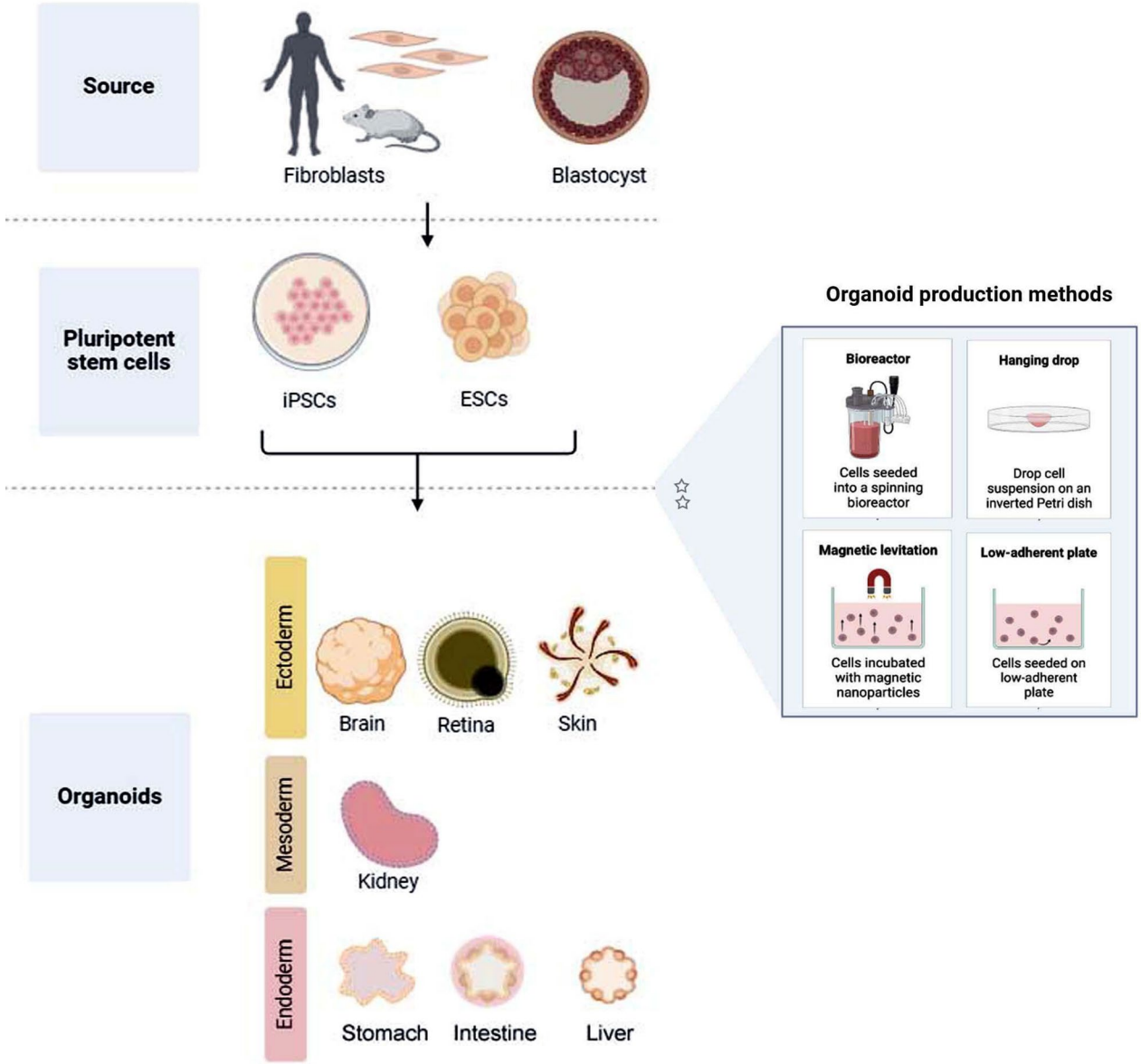

Fig. 1 Common steps in organoid generation from stem cells. Stars depict addition of a matrigel for common extracellular matrix (ECM), synthetic hydrogel (collagen or fibrin-based matrices) and growth factors like Wnt, Notch, BMP, EGF (depending on organ type) along with maturation factors such as HGF (hepatocyte growth factor) for liver organoids, and EGF for gut organoids. Cells are isolated using techniques such as enzymatic digestion, mechanical dissociation, or fluorescence-activated cell sorting (FACS) to enrich the desired cell population. Organoids are cultured for several weeks to ensure proper differentiation

modifying culture conditions [21]. Structurally, gut organoids feature a luminal region where apoptotic enterocytes and metabolites are expelled [52]. However, unlike the gut mucosa, where enterocytes face the external environment apically, organoids have an inverted polarity, with the apical side facing inward. Recent advancements have enabled the reversal of this polarity, allowing the apical surface to be exposed to the culture medium, thus facilitating better interaction with external stimuli [53].

Liver organoids are commonly cultured in Matrigel (comprising collagen, nestin, fibronectin etc.), supplemented with specific growth factors. They are generally maintained under normoxic conditions (21% O_2) but can also be adapted to hypoxic conditions to model certain pathological states [54]. Passaging is done every 7–14 days, depending on their growth rate, by dissociating them into smaller clusters or single cells and re-embedding them in fresh Matrigel. The pioneering work of Huch

et al. demonstrated the generation of liver organoids from *Lgr5*+hepatocytes, which possess both proliferative and differentiation potential [55]. Subsequent studies established hepatocyte-derived organoids from single mature hepatocytes in both mice and humans, exhibiting key hepatic functions and gene expression profiles similar to in vivo hepatocytes, with sustained proliferation for at least six months [56, 57]. Further advancements have led to the development of vascularized liver buds through the co-culture of human hepatic endoderm and stromal cells. These structures express early liver-specific markers such as alpha-fetoprotein and albumin and demonstrate drug metabolism capabilities [58, 59]. More recently, hepatobiliary organoids (exhibiting functions of both hepatocytes i.e., drug metabolism and albumin production and bile duct cells) have been established [60, 61]. Additionally, hepatocyte-like organoids resembling adult liver tissue have been successfully fabricated using organ-on-chip technology, further enhancing their potential for disease modeling and drug testing [61].

The development of organoids for specific applications, such as modeling various cancers or generating patient-derived organoids for precision oncology, requires optimized protocols that refine differentiation procedures and adjust culture conditions to better reflect disease complexity [62, 63]. This process involves multicellular self-organization and patterning, coordinated with tightly regulated differentiation, proliferation, and apoptosis, ultimately leading to the formation of mature, functional tissues [2]. For long-term maintenance, organoids are cultured with regular medium changes and passaging to sustain their viability and functionality [64]. Their characterization relies on various techniques, including immunohistochemistry, gene expression analysis, functional assays, and advanced imaging methods [65, 66]. In general, organoid production requires a multidisciplinary approach, integrating expertise from developmental biology, tissue engineering, stem cell biology, and bioinformatics. Over time, protocols have been continuously optimized and refined, evolving alongside our expanding understanding of organoid biology and the molecular mechanisms governing organ development and function.

Metabolic potential of organoids

The organoids can be used for metabolic assessment of chemicals (Fig. 2). In particular, liver organoids exhibit significant metabolic potential, serving as biofactories capable of reproducing detoxification and metabolic replication. When liver organoids are embedded in low stiffness gelatins and cultured under perfusion conditions, they can effectively metabolize ammonia, reflecting the metabolic activity observed in vivo [67]. They retain the ability to perform gluconeogenesis, a key metabolic function of the liver [68]. Furthermore, liver organoids exhibit metabolic stress responses to high glucose levels, showing mitochondrial membrane potential alterations, lipid accumulation, and reactive oxygen species formation, akin to in vivo conditions [69]. Liver organoids also effectively model metabolic diseases. Exposure to lactate, pyruvate, and octanoic acid replicates key features of fatty liver disease, including intracellular lipid accumulation and metabolic

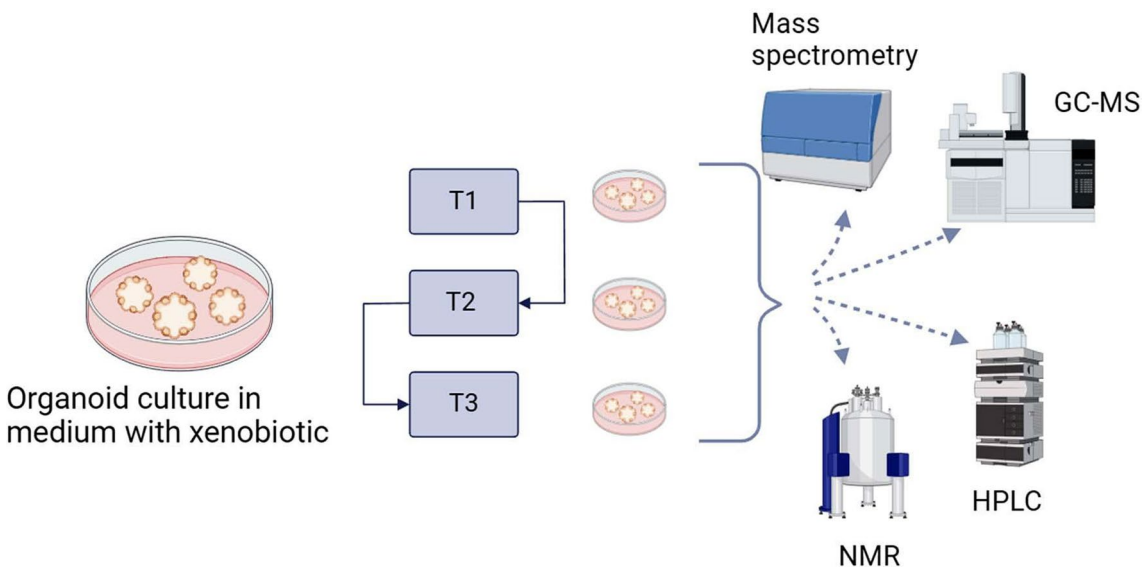

Fig. 2 Metabolic assessment of organoids at various time points (T1, T2, T3) using techniques such as GC–MS, LC–MS, and mass spectrometry could be used to determine metabolites under specific conditions and exposure to xenobiotics

dysregulation [70]. Their response to pharmacological agents further underscores their utility in drug metabolism studies. For instance, in a co-culture system, Docetaxel exposure resulted in varying survival rates among undifferentiated, differentiated, and cytochrome P450-induced differentiated organoids, demonstrating the significance of cytochrome induction in drug metabolism and toxicity [71]. At the molecular level, liver organoids express hepatic markers such as albumin (ALB), α-fetoprotein (AFP), CYP2C9, and CYP7A1, indicating differentiation towards hepatic function [72]. They have also proven effective in detecting drug-induced toxicity. An example is of indomethacin (Cmax: 8.38 μmol/L) and zileuton (Cmax: 13.12 μmol/L), which exhibited measurable cytotoxicity at concentrations of 10 μmol/L and 100 μmol/L, respectively. Collectively, these findings illustrate the metabolic plasticity of liver organoids, enabling them to respond dynamically to environmental cues and mimic pathological conditions. Their ability to replicate key metabolic and toxicological responses underscores their potential as powerful platforms for disease modeling and drug testing.

Gut or intestinal organoids also hold considerable promise for studying intestinal epithelial cell metabolism of xenobiotics and nutrient absorption. 3D co-culture intestinal organoid systems have been developed to explore glucose metabolism, with glucose uptake, utilization, and production by different cell types, providing insights into the complex metabolic processes occurring in the gut [73, 74]. A common finding across studies is that dietary factors impact the gut function [75]. It has been successfully demonstrated that high sucrose can lead to systemic changes in zinc distribution, with enhanced expression of the zinc transporter ZIP14 in the basolateral membrane of intestinal epithelial cells, which in turn causes tight junction dysregulation and increased permeability [76]. These findings emphasize how dietary components can directly influence gut barrier integrity and nutrient absorption. Additionally, research has demonstrated that gut organoids can simulate drug metabolism similarly to the human intestine. Induction of CYP3A4 and ABCB1 gene expression has been observed in the organoids, suggesting their capability to metabolize drugs similarly to the human intestine [77]. Hence, gut organoids can recapitulate the complex architecture and functions of the intestinal epithelium, providing a physiologically relevant system for investigating nutrient metabolism, drug absorption, and host-microbiome interactions. Recent advances in CRISPR/Cas9 and metabolic programming technologies have further expanded the potential of organoids for studying metabolism under chemical stress behavior [30].

Role in xenobiotic toxicology assessment

Xenobiotics are foreign chemical substances that are not naturally produced by an organism. They include a wide range of man-made and natural substances like pharmaceuticals, industrial chemicals, preservatives, artificial colors and flavors, plasticizers etc. [78]. Naturally produced chemicals in this category may include fungal, plant or bacterial toxins. High levels of metals like lead, mercury, and arsenic can be toxic and are considered xenobiotics when they enter the body at excessive amounts [79]. These toxins and contaminated food/water pose significant public health risks. However, studying human-specific responses and long-term effects of such toxins remains challenging [80]. Factors such as route of exposure, duration, and frequency can vary widely among individuals, making it difficult to establish dose–response relationships [81]. Biomonitoring data, which measures the presence of toxicants in biological samples, may not always reflect the internal dose or the target organ concentration, leading to uncertainties in risk assessment [82]. Recent advances have highlighted the potential of organoids as a more physiologically relevant and efficient tool for assessing the hazards and risks associated with xenobiotics. Compared to traditional in vitro experiments and animal models, organoids provide a more accurate representation of human-specific responses, making them a promising platform for studying toxicological effects and improving risk assessments (Table 2).

Studying the effects of toxicants on organoid development, gene expression, and signaling pathways provides valuable insights into the underlying mechanisms of toxicity [102]. Organoids offer potential advantages over traditional in vitro and animal models for environmental toxicology research and offer the ability to study the effects of environmental toxicants on specific organs, such as the liver, kidney, or brain. Here, we focus on gut and liver organoids due to their key roles in toxin absorption and detoxification. The intestinal epithelium is the first line of defense against many ingested toxins. Exposure of gut organoids to toxicants can serve as a model for identifying potential damage to the digestive system, such as compromised nutrient uptake and weakened barrier function [87]. Liver is the primary detox center of the body and metabolizes/eliminates xenobiotics [103]. Liver organoids allow researchers to study the impact of toxins on hepatotoxicity and provide valuable insights into liver-specific toxic effects [104]. Focusing on organ-specific toxicity using organoids creates a more targeted understanding of potential health risks. Moreover, organoids offer a unique opportunity to explore the cellular and molecular mechanisms that underlie these toxic effects, enhancing our ability to predict long-term health

Table 2 Examples of environmental toxins and drugs that could be possibly studied using gut and liver organoids. Possible endpoints and outcomes have also been listed

Serial No	Toxin Class	Example	Possible Endpoints to Study	Potential Outcomes
1	Environmental Pollutants	Polycyclic aromatic hydrocarbons [83], polychlorinated biphenyls [84], pesticides [85], Dioxins, food additives, plasticizers and microplastics, flame retardants, metals, nanoparticles, combustion products, personal care products etc. [86]	Absorption, metabolism, toxicity, barrier function disruption, morphological changes and cellular damage [87], gene expression [88]	Improved risk assessment of chemicals [89], identification of potential hazards [84], informing decisions about regulations and restrictions [90]
2	Prescription Medications	Antibiotics [91] (e.g., ciprofloxacin), antidepressants [92] (e.g., fluoxetine), NSAIDs [93] (e.g., ibuprofen)	Absorption, metabolism, efficacy, intestinal side effects [73] (e.g., diarrhea), drug-drug interactions [94]	Personalized medicine approaches [95], optimizing drug dosing regimens [96], identifying potential off-target effects, reducing reliance on animal testing [97]
3	Illicit Drugs	Cocaine, heroin [98, 99]	Absorption, metabolism, addictive potential, intestinal damage [100]	Evaluating potential harm to body, from chronic use [101]

consequences and refine environmental safety assessments. Here is an overview of these organoid systems and their potential applications in assessing toxin hazards and risks:

Liver organoids

Liver organoids express key metabolic enzymes and transporters, making them suitable for studying the metabolism and toxicity of chemicals [105]. They have been used to assess the hepatotoxicity of various compounds, including drugs and environmental pollutants.

Environmental toxin study

Different classes of toxins exhibit distinct toxicity profiles, and researchers have utilized liver organoids to investigate these effects by examining varying concentrations and elucidate their mechanisms through molecular pathways. Heavy metals such as lead, mercury, thallium, and glyphosate have been tested for their cytotoxic effects on liver organoids. Thallium has exhibited the highest toxicity, with an IC50 of 13.5 μM, followed by mercury (30.8 μM), glyphosate (10.53 mM), and lead (2.98 mM) [106]. These toxins induced significant cellular damage, as assessed by viability assays and ATP activity measurements. Per-and polyfluoroalkyl substances (PFAS) have also been investigated in liver organoids. The short-chain PFAS, such as heptafluorobutyric acid (HFBA) and pentafluoropropionic anhydride (PFPA), caused cytomorphological aberrations and enzyme disruption but did not induce acute toxicity or apoptosis [107]. In contrast, long-chain PFAS, including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), displayed EC50 values exceeding 650 μM and were associated with significant enzyme disruptions, caspase activation, and architectural disintegration at higher concentrations (500–1000 μM).

Emerging contaminants, such as microplastics, nanofibres and endocrine-disrupting chemicals have shown adverse effects on liver organoids. A diminished proliferative capacity of organoids in nanofiber (HYDROX) culture has been observed, with a significant improvement in the gene expression levels of drug-metabolizing enzymes [108]. Microplastics and tetrabromobisphenol A have shown no effect on healthy donor-derived organoids but ALD organoids have exhibited altered gene expression patterns related to liver sinusoidal endothelial cells (STAB2, LYVE1) and metabolic dysfunctions [109]. RNA sequencing further revealed enrichment of pathways associated with oxidative phosphorylation and cytoplasmic translation. Similarly, the impact of 1 μm polystyrene microplastics and bisphenol A has been studied on liver organoids at human internal exposure level. An increased hepatotoxicity due to co-exposure, leading to oxidative stress, lipid metabolism disruption, and epigenetic alterations, was observed [110]. The combined adverse effects of microplastics and bisphenol were synergistic, worsening hepatotoxicity and disrupting gene panels associated with various lipid metabolism processes, alongside the proteins HNF4A, CD36, ACC1, CPT1A, CYP2E1, ERα, and ERβ. Microplastics did not alter ERα or Erβ alone but co-exposure with bisphenol significantly and synergistically elevated ERα levels. A potential adverse outcome pathway predicting hepatic steatosis was proposed for the co-exposure. These findings underscore the metabolic health risks associated with co-exposure to microplastics and BPA, even at low doses. Further research has shown the effects of pristine polystyrene microplastics (pPS) and aged polystyrene microplastics (aPS) [111]. aPS exhibited higher cytotoxicity, inducing mitochondrial dysfunction, lipid peroxidation, and iron transport disruption. Elevated SLC7A11 and FTL expression indicated oxidative stress and iron metabolism dysregulation.

Drug related toxicity assessment

Drug-induced liver injury, and other adverse outcomes are a major contributor to late-stage clinical trial failures and post-marketing withdrawals [112]. Current preclinical testing relies on 2D cell cultures and animal models with known limitations in predicting human-specific toxicity. Liver organoids offer an advanced platform to evaluate drug-induced toxicity with enhanced physiological relevance. Human liver organoids have been used to investigate the biological responses and hepatotoxicity of pharmaceutical compounds. The antiepileptic drug magnesium oxide and the nanoparticle valproate have indicated reduced cell viability, decreased ATP production, and increased reactive oxygen species in liver organoids [84, 113]. Exposure to troglitazone, amiodarone, and acetaminophen have shown upregulation in the drug metabolizing enzymatic activity [108, 114]. Wu et al. demonstrated that liver organoids exposed to acetaminophen, fialuridine, methotrexate, and fasiglifam exhibited phenotypic changes concordant with human clinical data, highlighting their predictive potential in drug safety testing [115]. Chronic exposure to troglitazone, trovafloxacin, acetaminophen, and steatosis-inducing agents such as oleate and palmitate resulted in inflammation, hepatotoxicity, and hepatic steatosis [116]. A multiplexed readout system to measure viability, cholestatic, and mitochondrial toxicity of drugs for mechanistic studies has also been developed [72] which demonstrates that CYP2C9*2 mutation is predisposed to Bosentan-induced cholestasism, underscoring the potential of liver organoids in precision medicine and personalized drug screening.

Furthermore, IC50 and benchmark doses for various drugs, including 5-Fluorouracil, carbofuran, valproate, paracetamol, troglitazone, and voriconazole have been assessed for potential hepatotoxicity in fatty liver models. Notably, troglitazone exhibited cytotoxicity in liver organoids but not in 2D cultures, demonstrating the superior sensitivity of organoid models [117]. The effects of triptolide, honokiol, and doxorubicin in HepG2 liver cell organoids have also been explored [118]. Triptolide and honokiol reversed the epithelial-mesenchymal transition process but doxorubicin did not, reinforcing the efficacy of tumor organoid-like models in screening antitumor agents. These findings collectively highlight the advantages of liver organoids in assessing drug-induced toxicity, providing a human-relevant platform for hepatotoxicity studies, and advancing drug safety evaluations.

Gut organoids

Gut organoids are derived from intestinal stem cells and can form the various cell types found in the intestinal epithelium, including absorptive enterocytes, goblet cells, and Paneth cells [119]. These organoids can be used to study the effects of environmental toxicants on intestinal barrier function, nutrient absorption, and immune responses.

Environmental toxin study

Despite lacking enteric nerve and immune cells, intestinal or gut organoids have been instrumental in various studies due to stable cell culture conditions [52]. Gut organoids are particularly relevant for assessing the toxicity of ingested toxicants, such as heavy metals and pesticides. Metal toxicity analysis with repeated administration of cadmium, lead, hexavalent chromium, and inorganic trivalent arsenic has shown a reduced cell viability and differentiation along with apoptosis induction, mucus production dysfunction, and damage to the epithelial barrier [120]. The morphological features induced by each metal were specific and mediated by distinct signaling pathways, including Wnt, bone morphogenetic protein, apoptosis induction, and Notch pathways.

Gut organoids have been instrumental in studying the genotoxicity and cytotoxicity of various compounds. For instance, exposure to the carcinogen benzo[a]pyrene induced cytotoxicity, upregulated xenobiotic-metabolizing enzyme genes (CYP1A1, NQO1), and activated the DNA damage response pathway, leading to the formation of DNA adducts and metabolites [21, 102]. Studies on dietary compounds have demonstrated diverse effects. Caffeic acid inhibited organoid growth in a concentration-dependent manner, while curcumin produced variable effects, and vitamin C showed no impact [121]. Long-term exposure to the sweetener rebaudioside A upregulated enteroendocrine-specific markers, including chromogranin A, glucagon, Pyy, and cholecystokinin [122]. Similarly, alcohol at even 0.2% significantly inhibited small intestinal organoid growth [123].

Toxicity assessments have revealed the impact of various nanoparticles and food additives. High concentrations of niobium carbide (Nb_2C) nanosheets suppressed organoid growth [124] while food-grade titanium dioxide (TiO_2) exposure altered differentiation markers such as muc2, vilin 1, and chromogranin A in a dose-dependent manner [125]. Further, fg-TiO_2 induced apoptosis, genotoxicity, and a decline in antimicrobial peptide and tight junction-related gene expression, highlighting its potential impact on intestinal barrier integrity. Sunset yellow, a common food additive, disrupted cell proliferation and differentiation while inducing oxidative and ER stress [126]. Additionally, graphene quantum dots reduced organoid size [127]. Beyond toxicity studies, gut organoids have been employed to assess apoptosis in intestinal epithelial cells exposed to 5-Fluorouracil, using microscopic and colorimetric analyses [126]. Their predictive accuracy has also been validated in evaluating ricin transport and toxicity in vivo [128].

Drug related toxicity assessment

Early-life antibiotic exposure has been shown to impact epithelial cell maturation and barrier function. Amoxicillin, vancomycin, and metronidazole have been observed to decrease intestinal permeability, altered epithelial defense, transepithelial sensing capacity and reduced the number of specialized vacuolated cells essential for milk macromolecule absorption in neonatal mice [129]. Rifampicin treatment of intestinal organoid epithelial cells under CYP3A4-induced conditions have led to a decline in N-(4-hydroxyphenyl)retinamide cytotoxicity [130]. Similarly, intestinal and gut epithelial cell-derived organoids have been used to investigate the effect of bacitracin against *Clostridium difficile* Toxin B (TcdB) [131]. It inhibits TcdB by preventing its pH-dependent transport across membranes, thereby blocking its interaction with Rac1 in human epithelial cells. Organoids have also shown strong predictive capabilities for gastrointestinal toxicity. A human gut organoid model demonstrated 90% accuracy in predicting drug-induced gastrointestinal toxicity across a reference set of 31 drugs, surpassing traditional rodent models [87]. Drug treatment altered the expression of key gut epithelial markers such as lysozyme, chromogranin A, mucin, and sucrase-isomaltase. The study highlighted the importance of incorporating toxicologically relevant dosing schedules, including daily drug administration and washout periods, to enhance assay predictability. Organoid models have also contributed to infectious disease research and

antiviral drug evaluation. Gut organoids susceptible to SARS-CoV-2 infection have shown that viral infection led to significant organoid deterioration except in goblet cells lacking ACE2 expression [132]. Remdesivir reduced SARS-CoV-2 infection by 86% at 500 nM and nearly abolished it at 5 μM. However, its potency was lower in organoids than in monolayer cell cultures, highlighting the importance of model selection in toxicology assessments.

Cystic fibrosis patient-derived organoids have been used to assess drug responsiveness. Forskolin-induced swelling in these organoids is entirely dependent on CFTR mutations, reflecting clinical therapy outcomes [133]. Additionally, organoids have been employed to assess antiviral drug efficacy. Masmoudi et al. have tested enviroxime, rupintrivir, and 2′-C-methylcytidine (2′CMC) against enterovirus 71 [134]. Organoids exhibited greater sensitivity to infection and drug treatment compared to conventional cell lines. However, 2′CMC displayed a poor selectivity index, failing to achieve 100% viability even at the highest tested concentration, suggesting potential low-grade toxicity to host cells. These findings reinforce the value of gut organoids as physiologically relevant models for drug toxicity assessment, offering improved predictive accuracy compared to traditional in vitro and in vivo models.

Limitations

In spite of several advantages, organoids have several limitations as well. Even though they mimic organ structures and functions to some extent, they fall short of replicating the complexity of the human body [135, 136]. They may not fully recapitulate the diversity and interactions found in vivo, often resembling developmental stages of organs rather than fully matured tissues [137]. This immaturity can affect their response to toxicants, as mature tissues may exhibit different sensitivities or detoxification mechanisms. Additionally, organoid cultures can display significant variability between batches and within the same batch, posing challenges for drawing consistent conclusions from experiments. Limited lifespan in culture restricts long-term toxicological studies, and the establishment and maintenance of organoid cultures can be costly and resource-intensive, requiring specialized equipment, reagents, and expertise [138].

Furthermore, the absence of vascularization in organoids hinders nutrient and oxygen availability, as blood arteries in vivo exhibit complex structures with variations in size, layers, and constituent cell types [139]. Although generating human blood vessels in vitro is feasible, achieving organoid vascularization remains challenging but is being worked upon in addition to standardization of protocols as they are lacking at the moment and contribute to variability between studies and hindering result comparison across different laboratories. Another limitation in organoid culture systems is the discrepancy between physiological oxygen levels and those maintained in standard laboratory conditions [140, 141]. Gut and liver experience varying degrees of hypoxia, particularly in regions like the intestinal crypts and periportal zones of the liver [142]. These low-oxygen environments play a crucial role in maintaining tissue homeostasis, regulating cellular metabolism, stem cell function, and responses to injury or toxins. In contrast, most organoid cultures are maintained under normoxic conditions (21% O_2), which do not accurately replicate the oxygen gradients present in vivo [143]. This discrepancy can lead to alterations in cellular behavior, gene expression, and metabolic activity, potentially limiting the translational relevance of organoid-based studies. Hypoxia-inducible factors (HIFs) and other oxygen-sensitive pathways may not be adequately activated under normoxic conditions [144, 145]. Therefore, organoid models may show altered responses to toxins and xenobiotics under these conditions, as these pathways affect drug metabolism, oxidative stress, and cellular repair mechanisms. To address this limitation, researchers have explored hypoxia-mimicking culture systems and specialized bioreactors designed to maintain physiologically relevant oxygen levels [146, 147]. However, these approaches are not yet standardized and introduce additional challenges, such as variability in oxygen gradients and potential impacts on organoid viability. Future studies should focus on optimizing these systems to better integrate hypoxic conditions, thereby enhancing the physiological relevance of organoid models. Research efforts are also desired to improve organoid culture techniques, enhance their physiological relevance, and validate their utility for toxicological studies.

Future perspective

Organoids are advantageous in environmental toxicology research due to their ability to be cultured in microplates, enabling high-throughput screening of multiple toxicants and concentrations simultaneously [148]. This approach enhances research efficiency and cost-effectiveness by expediting the identification of hazardous compounds and prioritizing them for further investigation. Moreover, organoids closely mimic native tissue architecture and function, providing increased translational relevance by utilizing human-derived cells to offer more accurate insights into human responses to environmental toxins. Beyond toxicity assessment, organoids show promise in reducing reliance on animal testing, offering more human-relevant models and yielding crucial data for evaluating the safety of environmental chemicals [149]. As organoid technology advances, one of the most

Table 3 Structured comparison of organoids, assembloids, and organ-on-chip technologies based on their capabilities

Feature	Organoids	Assembloids	Organ-on-Chip	References
Complexity	Moderate; mimics single organ/tissue types	High; combines multiple organoids to model tissue-tissue interactions	Moderate to high; mimics physiological conditions with mechanical cues	[150, 151]
Physiological Relevance	High for single-organ systems; limited for multi-organ interactions	High for studying inter-organ interactions and developmental processes	High for mimicking mechanical and biochemical cues of human physiology	[152–155]
Scalability	Limited scalability due to variability in size and structure	Limited scalability; complex to assemble and maintain	High scalability; compatible with high-throughput screening	[151, 154, 156–158]
Throughput	Low to moderate; labor-intensive and time-consuming	Low; requires advanced techniques for assembly and maintenance	High; suitable for automated and parallelized experiments	[155, 156, 159–161]
Cost	Moderate; requires specialized culture conditions	High; complex assembly and maintenance increase costs	High initial cost; lower operational costs for large-scale studies	[150, 155, 157, 161, 162]
Applications	Disease modeling, drug screening, developmental biology	Studying inter-organ interactions, neurodevelopmental processes	Drug testing, toxicity screening, disease modeling, personalized medicine	[150, 155, 157, 161, 163, 164]
Limitations	Limited vascularization, variability in size and structure	Technically challenging to assemble, limited long-term stability	Limited complexity in mimicking multi-organ systems, requires expertise	[154, 161, 164–166]
Projected Market (2023–2030)	$2.5 billion by 2030 (CAGR: 21.5%)	Emerging field; no specific market projection yet (subset of organoid market)	$1.6 billion by 2030 (CAGR: 36.7%)	[167] https://www.marketsandmarkets.com/Market-Reports/organs-on-chips-market-144117291.html; accessed 9 February 2025

promising developments is the emergence of assembloids (Table 3), which integrates multiple organoid types or incorporates additional cell populations such as immune, endothelial, or neural cells to better replicate in vivo tissue interactions [12]. Assembloids enhance physiological relevance by enabling the study of intercellular communication, vascularization, and systemic toxicity, which are often absent in conventional organoid models [84]. However, their development presents challenges, including precise control over cell ratios, spatial organization, and culture conditions to ensure functional maturation. Additionally, issues of scalability and reproducibility remain critical due to biological variability [12]. Emerging technologies are being explored to refine assembloid systems and improve their standardization.

Looking ahead, the organoid-on-chip approach, which incorporates microfluidic technology, holds promise for recreating near-physiological conditions, particularly in gut and liver organoids. Culturing organoids within interconnected microfluidic platforms allows for dynamic interactions between multiple organ systems, mimicking real-life scenarios such as the effect of liver metabolism on heart function. This innovation enables earlier detection of potential toxic effects, reducing reliance on animal testing. For instance, a double-layered microfluidic chip has been developed to create an intestine-liver model, facilitating investigations into drug absorption, transport, and metabolism [168]. By enabling multi-organ toxicity assessments, this technology provides a comprehensive understanding of adverse effects early in drug development. Moreover, organoids derived from a patient's cells hold potential for predicting individual responses to xenobiotics, leading to personalized risk assessment and treatment strategies. Moreover, advances in genetic engineering, such as the use of CRISPR/Cas9 technology, allow for the creation of organoid models with specific mutations or disease phenotypes, providing insights into how genetic factors influence the toxicological response. However, as these models become more widely adopted, ethical considerations surrounding the use of human-derived organoids in research must also be addressed [37].

Some of the challenges related to the lack of mature tissue organization and vascularization in organoid models are actively being addressed, offering hope for more accurate and reliable systems in toxicology and drug testing. One of the critical limitations, particularly in liver organoids, is the persistence of fetal-like hepatocytes, which exhibit limited functionality, such as suboptimal cytochrome P450 enzyme activity essential for drug metabolism. However, ongoing research is focused on refining culture conditions and incorporating advanced bioengineering techniques to enhance the maturation of hepatocytes, ensuring they replicate the metabolic capabilities of adult liver tissue. For example, the use of bioreactors and vascularization strategies, including the integration of endothelial cells and blood vessel-like structures within liver organoids, is progressing rapidly. These advancements will enable a more comprehensive understanding of systemic toxicity and better prediction of drug interactions and metabolic profiles in a human-specific context.

Similarly, in gut organoids, the inability to fully replicate the gut microbiome and the complex interactions between gut epithelial cells and immune cells remains a challenge. However, future efforts are directed towards creating more sophisticated models that incorporate microbial communities and simulate the immune responses observed in the human gut. With the development of organ-on-a-chip technologies and improved co-culture systems, it is becoming increasingly possible to mimic the dynamic microenvironment of gut, including its barrier function and immune system interactions. These innovations are expected to greatly enhance the ability of gut organoids to model real-world exposure scenarios, providing a more accurate representation of how environmental toxins, pharmaceuticals, and dietary compounds interact with human gastrointestinal tissues. The organoid market is projected to grow significantly due to their applications in drug discovery, personalized medicine, and disease modeling. The CAGR (Compound Annual Growth Rate) of 21.5% reflects strong demand (Table 3). As a newer and more specialized technology, assembloids do not yet have a separate market projection. They are often considered part of the broader organoid market. Organ-on-chip technology is expected to grow at a higher CAGR (36.7%) due to its scalability, high-throughput capabilities, and increasing adoption in pharmaceutical and biotechnology industries.

Conclusion

In conclusion, organoids offer several advantages over traditional in vitro and animal models for chemical toxicology research, including improved physiological relevance, personalized responses, reduced animal use, high-throughput screening, organ-specific toxicity assessment, mechanistic insights, and translational potential. Both gut and liver organoids are valuable tools in research, but their immature states often hinder their full potential. Hence, despite their many advantages, organoids also present challenges and limitations, including variability in development and function, limited lifespan in culture, and the absence of vascularization and immune system representation. Addressing these limitations will be crucial for further advancing the utility of organoids in toxicology research. Future directions

for research should focus on strategies for improving organoid complexity and functionality, integration with microfluidic technology, regulatory acceptance of organoid-based toxicology data, and ethical considerations surrounding the use of human-derived organoids. These advancements are expected to overcome current limitations, providing more sophisticated models for studying systemic toxicity, drug metabolism, and human-specific disease mechanisms in the near future.

Acknowledgements
The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

Author contributions
All authors read and approved the final manuscript. SMA conceived, designed, conducted, wrote and edited the review.

Funding
This study was funded by the Deanship of Scientific Research, Qassim University, KSA.

Data availability
Not applicable.

Declarations

Competing interest
The author declares no conflict of interest.

Received: 15 November 2024 Accepted: 4 March 2025
Published online: 26 March 2025

References

1. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P, Vries R, Peters PJ, Clevers H. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology. 2015;148(1):126-136.e6. https://doi.org/10.1053/j.gastro.2014.09.042.
2. Brassard JA, Lutolf MP. Engineering stem cell self-organization to build better organoids. Cell Stem Cell. 2019;24(6):860–76.
3. Rauth S, Karmakar S, Batra SK, Ponnusamy MP. Recent advances in organoid development and applications in disease modeling. Biochim et Biophys Acta-Rev Cancer. 2021;1875(2): 188527.
4. Yang S, Hu H, Kung H, Zou R, Dai Y, Hu Y, et al. Organoids: The current status and biomedical applications. MedComm. 2023;4(3): e274.
5. Ritter L, Arbuckle TE. Can exposure characterization explain concurrence or discordance between toxicology and epidemiology? Toxicol Sci. 2007;97(2):241–52.
6. Ockleford C, Adriaanse P, Berny P, Brock T, et al. Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects.' EFSA J. 2017. https://doi.org/10.2903/j.efsa.2017.5007.
7. Gamble JF. PM2. 5 and mortality in long-term prospective cohort studies: cause-effect or statistical associations? Environ Health Perspect. 1998;106(9):535–49.
8. Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, et al. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health, Part B. 2008;11(5–6):373–517.
9. Raies AB, Bajic VB. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip Rev: Comput Mol Sci. 2016;6(2):147–72.
10. Krewski D, Acosta D Jr, Andersen M, Anderson H, Bailar JC III, Boekelheide K, et al. Toxicity testing in the 21st century: a vision and a strategy. J Toxicol Environ Health, Part B. 2010;13(2–4):51–138.
11. Van Norman GA. Limitations of animal studies for predicting toxicity in clinical trials: Part 2: Potential alternatives to the use of animals in preclinical trials. Basic Trans Sci. 2020;5(4):387–97.
12. Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K, Lin L, et al. Organoids. Nat Rev Methods Primers. 2022;2(1):94.
13. Astashkina A, Mann B, Grainger DW. A critical evaluation of in vitro cell culture models for high-throughput drug screening and toxicity. Pharmacol Ther. 2012;134(1):82–106.
14. Nabi SU, Ali SI, Rather MA, Sheikh WM, Altaf M, Singh H, et al. Organoids: A new approach in toxicity testing of nanotherapeutics. J Appl Toxicol. 2022;42(1):52–72.
15. Varani J, McClintock SD, Aslam MN. Cell-matrix interactions contribute to barrier function in human colon organoids. Front Med. 2022;9: 838975.
16. Zheng L, Zhan Y, Wang C, Fan Q, Sun D, Li Y, et al. Technological advances and challenges in constructing complex gut organoid systems. Front Cell Dev Biol. 2024;12:1432744.
17. Kwon O, Han T-S, Son M-Y. Intestinal morphogenesis in development, regeneration, and disease: the potential utility of intestinal organoids for studying compartmentalization of the crypt-villus structure. Front Dev Biol. 2020;8: 593969.
18. Peng WC, Kraaier LJ, Kluiver TA. Hepatocyte organoids and cell transplantation: What the future holds. Exp Mol Med. 2021;53(10):1512–28.
19. Augustyniak J, Bertero A, Coccini T, Baderna D, Buzanska L, Caloni F. Organoids are promising tools for species-specific in vitro toxicological studies. J Appl Toxicol. 2019;39(12):1610–22.
20. Matsui T, Shinozawa T. Human organoids for predictive toxicology research and drug development. Front Genet. 2021;12: 767621.
21. Caipa Garcia AL, Arlt VM, Phillips DH. Organoids for toxicology and genetic toxicology: applications with drugs and prospects for environmental carcinogenesis. Mutagenesis. 2022;37(2):143–54.
22. Lou Y-R, Leung AW. Next generation organoids for biomedical research and applications. Biotechnol Adv. 2018;36(1):132–49.
23. Belair DG, Wolf CJ, Moorefield SD, Wood C, Becker C, Abbott BD. A three-dimensional organoid culture model to assess the influence of chemicals on morphogenetic fusion. Toxicol Sci. 2018;166(2):394–408.
24. Xu C, Ma H, Gao F, Zhang C, Hu W, Jia Y, et al. Screening of organophosphate flame retardants with placentation-disrupting effects in human trophoblast organoid model and characterization of adverse pregnancy outcomes in mice. Environ Health Perspect. 2022;130(5): 057002.
25. Zhang Y, Liu K, He H, Xiao H, Fang Z, Chen X, et al. Innovative explorations: unveiling the potential of organoids for investigating environmental pollutant exposure. Environ Sci Pollut Res. 2024;31(11):16256–73.
26. Ranga A, Gjorevski N, Lutolf MP. Drug discovery through stem cell-based organoid models. Adv Drug Deliv Rev. 2014;69:19–28.
27. Hoang P, Ma Z. Biomaterial-guided stem cell organoid engineering for modeling development and diseases. Acta Biomater. 2021;132:23–36.
28. Sahu S, Sharan SK. Translating embryogenesis to generate organoids: novel approaches to personalized medicine. Iscience. 2020;23(9):101485.
29. Ghosh D, Mehta N, Patil A, Sengupta J. Ethical issues in biomedical use of human embryonic stem cells (hESCs). J Reprod Health Med. 2016;2:S37–47.
30. Hu W, Lazar MA. Modelling metabolic diseases and drug response using stem cells and organoids. Nat Rev Endocrinol. 2022;18(12):744–59.
31. Kruczek K, Swaroop A. Pluripotent stem cell-derived retinal organoids for disease modeling and development of therapies. Stem cells Cloning: Adv Appl. 2020;38(10):1206–15.
32. Tian Z, Yu T, Liu J, Wang T, Higuchi A. Introduction to stem cells. Prog Mol Biol Trans Sci. 2023;199:3–32.
33. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res. 2007;100(9):1249–60.

34. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. Nat Rev Genet. 2018;19(11):671–87.
35. Asatrian G, Pham D, Hardy WR, James AW, Peault B. Stem cell technology for bone regeneration: current status and potential applications. Stem Cells Cloning: Advances Applications. 2015;39–48.
36. Qian S, Mao J, Liu Z, Zhao B, Zhao Q, Lu B, et al. Stem cells for organoids. Smart Med. 2022;1(1): e20220007.
37. De Jongh D, Massey EK, Bunnik EM. Organoids: a systematic review of ethical issues. Stem Cell Res Therapy. 2022;13(1):337.
38. Park I-H, Lerou PH, Zhao R, Huo H, Daley GQ. Generation of human-induced pluripotent stem cells. Nat Protoc. 2008;3(7):1180–6.
39. Charron D, Suberbielle-Boissel C, Al-Daccak R. Immunogenicity and allogenicity: A challenge of stem cell therapy. J Cardiovasc Transl Res. 2009;2:130–8.
40. Fong IW. Immune reconstitution inflammatory syndrome and paradoxical reaction. In: Fong IW, editor. Current Trends and Concerns in Infectious Diseases. Cham: Springer International Publishing; 2020. p. 31–58. https://doi.org/10.1007/978-3-030-36966-8_2.
41. Marchini A, Gelain F. Synthetic scaffolds for 3D cell cultures and organoids: applications in regenerative medicine. Crit Rev Biotechnol. 2022;42(3):468–86.
42. Boylin K, Aquino GV, Purdon M, Abedi K, Kasendra M, Barrile R. Basic models to advanced systems: harnessing the power of organoids-based microphysiological models of the human brain. Biofabrication. 2024;16(3): 032007.
43. Mansouri M, Leipzig ND. Advances in removing mass transport limitations for more physiologically relevant in vitro 3D cell constructs. Biophys Rev. 2021. https://doi.org/10.1063/5.0048837.
44. Nwokoye PN, Abilez OJ. Bioengineering methods for vascularizing organoids. Cell Rep Methods. 2024;4(6):100779. https://doi.org/10.1016/j.crmeth.2024.100779.
45. Skarne N, D'Souza RC, Palethorpe HM, Bradbrook KA, Gomez GA, Day BW. Personalising glioblastoma medicine: explant organoid applications, challenges and future perspectives. Acta Neuropathol Commun. 2025;13(1):6.
46. Suong DNA, Imamura K, Kato Y, Inoue H. Design of neural organoids engineered by mechanical forces. IBRO Neurosci Rep. 2024;16:190–5.
47. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, et al. Lgr5+ ve stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell. 2010;6(1):25–36.
48. Schlaermann P, Toelle B, Berger H, Schmidt SC, Glanemann M, Ordemann J, et al. A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. Gut. 2016;65(2):202–13.
49. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van Den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141(5):1762–72.
50. Kakni P, Jutten B, Carvalho DTO, Penders J, Truckenmüller R, Habibovic P, Giselbrecht S. Hypoxia-tolerant apical-out intestinal organoids to model host-microbiome interactions. J Tissue Eng. 2023. https://doi.org/10.1177/20417314221149208.
51. Lukovac S, Roeselers G. Intestinal crypt organoids as experimental models. Verhoeckx KC, P.; López-Expósito, I.; Kleiveland, C.; Lea, T.; Mackie, A.; Requena, T.; Swiatecka, D.; Wichers, H., editor. Switzerland: Springer International Publishing; 2015.
52. Gómez DP, Boudreau F. Organoids and their use in modeling gut epithelial cell lineage differentiation and barrier properties during intestinal diseases. Front Cell Deve Biol. 2021;9: 732137.
53. Margalef-Català M, Li X, Mah AT, Kuo CJ, Monack DM, Amieva MR. Controlling epithelial polarity: a human enteroid model for host-pathogen interactions. Cell Rep. 2019;26(9):2509–20.
54. Vicente P, Almeida JI, Crespo IE, Virgolini N, Isidro IA, Calleja-Cervantes ME, et al. Oxygen control in bioreactor drives high yield production of functional hiPSC-like hepatocytes for advanced liver disease modelling. Sci Rep. 2024;14(1):24599.
55. Huch M, Boj SF, Clevers H. Lgr5+ liver stem cells, hepatic organoids and regenerative medicine. Regen Med. 2013;8(4):385–7.
56. Hu H, Gehart H, Artegiani B, LÖpez-Iglesias C, Dekkers F, Basak O, et al. Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell. 2018;175(6):1591–606.
57. Zhu X, Zhang B, He Y, Bao J. Liver organoids: Formation strategies and biomedical applications. Tissue Eng Regen Med. 2021;18(4):573–85.
58. Abbasalizadeh S, Babaee S, Kowsari-Esfahan R, Mazidi Z, Shi Y, Wainer J, et al. Continuous production of highly functional vascularized hepatobiliary organoids from human pluripotent stem cells using a scalable microfluidic platform. Adv Func Mater. 2023;33(49):2210233.
59. Hosseini V, Maroufi NF, Saghati S, Asadi N, Darabi M, Ahmad SNS, et al. Current progress in hepatic tissue regeneration by tissue engineering. J Transl Med. 2019;17:1–24.
60. Marsee A, Roos FJ, Verstegen MM, Roos F, Verstegen M, Clevers H, et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. Cell Stem Cell. 2021;28(5):816–32.
61. Tanimizu N, Ichinohe N, Sasaki Y, Itoh T, Sudo R, Yamaguchi T, et al. Generation of functional liver organoids on combining hepatocytes and cholangiocytes with hepatobiliary connections ex vivo. Nat Commun. 2021;12(1):3390.
62. Pernik MN, Bird CE, Traylor JI, Shi DD, Richardson TE, McBrayer SK, et al. Patient-derived cancer organoids for precision oncology treatment. J Personal Med. 2021;11(5):423.
63. Skala MC, Deming DA, Kratz JD. Technologies to assess drug response and heterogeneity in patient-derived cancer organoids. Annu Rev Biomed Eng. 2022;24(1):157–77.
64. Mun SJ, Hong Y-H, Ahn H-S, Ryu J-S, Chung K-S, Son MJ. Long-term expansion of functional human pluripotent stem cell-derived hepatic organoids. Int J Stem Cells. 2020;13(2):279–86.
65. Kim H-Y, Charton C, Shim JH, Lim SY, Kim J, Lee S, et al. Patient-derived organoids recapitulate pathological intrinsic and phenotypic features of fibrous dysplasia. Cells. 2024;13(9):729.
66. Na JC, Kim J-H, Kim SY, Gu Y-R, Jun D-Y, Lee HH, et al. Establishment of patient-derived three-dimensional organoid culture in renal cell carcinoma. Investig Clin Urol. 2020;61(2):216–23.
67. Bernal PN, Bouwmeester M, Madrid-Wolff J, Falandt M, Florczak S, Rodriguez NG, et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories. Adv Mater. 2022;34(15):2110054.
68. Gamboa CM, Wang Y, Xu H, Kalemba K, Wondisford FE, Sabaawy HE. Optimized 3D culture of hepatic cells for liver organoid metabolic assays. Cells. 2021;10(12):3280.
69. Lu Y, Zhang G, Shen C, Uygun K, Yarmush ML, Meng Q. A novel 3D liver organoid system for elucidation of hepatic glucose metabolism. Biotechnol Bioeng. 2012;109(2):595–604.
70. Wang L, Li M, Yu B, Shi S, Liu J, Zhang R, et al. Recapitulating lipid accumulation and related metabolic dysregulation in human liver-derived organoids. J Mol Med. 2022;100(3):471–84.
71. Park E, Kim HK, Jee J, Hahn S, Jeong S, Yoo J. Development of organoid-based drug metabolism model. Toxicol Appl Pharmacol. 2019;385: 114790.
72. Shinozawa T, Kimura M, Cai Y, Saiki N, Yoneyama Y, Ouchi R, et al. High-fidelity drug-induced liver injury screen using human pluripotent stem cell–derived organoids. Gastroenterology. 2021;160(3):831–46.
73. Zietek T, Boomgaarden WA, Rath E. Drug screening, oral bioavailability and regulatory aspects: a need for human organoids. Pharmaceutics. 2021;13(8):1280.
74. Nie J, Liao W, Zhang Z, Zhang M, Wen Y, Capanoglu E, et al. A 3D co-culture intestinal organoid system for exploring glucose metabolism. Curr Res Food Sci. 2023;6: 100402.
75. Zhang D, Zhou X, Zhou W, Cui SW, Nie S. Intestinal organoids: A thriving and powerful tool for investigating dietary nutrients-intestinal homeostasis axis. Food Res Int. 2023;172: 113109.
76. Mitchell SB, Hung Y-H, Thorn TL, Zou J, Baser F, Gulec S, et al. Sucrose-induced hyperglycemia dysregulates intestinal zinc metabolism and integrity: risk factors for chronic diseases. Front Nutr. 2023;10:1220533.
77. Sasaki K, Inoue M, Machida M, Kawasaki T, Tsuruta S, Uchida H, et al. Human pluripotent stem cell-derived organoids as a model of intestinal xenobiotic metabolism. StemJournal. 2021;3(1):1–10.
78. Iovdijová A, Bencko V. Potential risk of exposure to selected xenobiotic residues and their fate in the food chain-Part. I: Classification of xenobiotics. Ann Agric Environ Med. 2010;17(2):183–92.
79. Rahman Z, Singh VP. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead

(Pb)) on the total environment: an overview. Environ Monit Assess. 2019;191:1–21.
80. Scheer N, Wilson ID. A comparison between genetically humanized and chimeric liver humanized mouse models for studies in drug metabolism and toxicity. Drug Discov Today. 2016;21(2):250–63.
81. Zeise L, Wilson R, Crouch E. Dose-response relationships for carcinogens: a review. Environ Health Perspect. 1987;73:259–306.
82. Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L, et al. The use of biomonitoring data in exposure and human health risk assessments. Environ Health Perspect. 2006;114(11):1755–62.
83. Gearhart-Serna LM, Davis JB, Jolly MK, Jayasundara N, Sauer SJ, Di Giulio RT, et al. A polycyclic aromatic hydrocarbon-enriched environmental chemical mixture enhances AhR, antiapoptotic signaling and a proliferative phenotype in breast cancer cells. Carcinogenesis. 2020;41(12):1648–59.
84. Li M, Gong J, Gao L, Zou T, Kang J, Xu H. Advanced human developmental toxicity and teratogenicity assessment using human organoid models. Ecotoxicol Environ Safety. 2022;235: 113429.
85. Wang Y, Yin N, Yang R, Zhao M, Li S, Zhang S, et al. Development of a simplified human embryonic stem cell-based retinal pre-organoid model for toxicity evaluations of common pollutants. Cutan Ocular Toxicol. 2023;42(4):264–72.
86. Wang Z, Chen S, Guo Y, Zhang R, Zhang Q, Jiang X, et al. Intestinal carcinogenicity screening of environmental pollutants using organoid-based cell transformation assay. Arch Toxicol. 2024;98(6):1937–51.
87. Markus J, Landry T, Stevens Z, Scott H, Llanos P, Debatis M, et al. Human small intestinal organotypic culture model for drug permeation, inflammation, and toxicity assays. In Vitro Cell Dev Biol-Anim. 2021;57:160–73.
88. Michalopoulos GK, Bowen WC, Mulè K, Luo J. HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. Gene Expr. 2018;11(2):55.
89. Nitsche KS, Müller I, Malcomber S, Carmichael PL, Bouwmeester H. Implementing organ-on-chip in a next-generation risk assessment of chemicals: a review. Arch Toxicol. 2022;96(3):711–41.
90. Anklam E, Bahl MI, Ball R, Beger RD, Cohen J, Fitzpatrick S, et al. Emerging technologies and their impact on regulatory science. Exp Biol Med. 2022;247(1):1–75.
91. Bing W, Haney EF, Akhoundsadegh N, Pletzer D, Trimble MJ, Adriaans AE, et al. Human organoid biofilm model for assessing antibiofilm activity of novel agents. npj Biofilms Microbiomes. 2021. https://doi.org/10.1038/s41522-020-00182-4.
92. Marinho LSR, Chiarantin GMD, Ikebara JM, Cardoso DS, de Lima-Vasconcellos TH, Higa GSV, et al., editors. The impact of antidepressants on human neurodevelopment: brain organoids as experimental tools. Seminars in Cell & Developmental Biology; 2023: Elsevier.
93. Lee GS, Cody AS, Johnson KC, Zhao H, Odelberg SJ, Li DY, et al. Estrogen enhances female small intestine epithelial organoid regeneration. J Bio-X Res. 2019;2(1):9–15.
94. Ingelman-Sundberg M, Lauschke VM. Human liver spheroids in chemically defined conditions for studies of gene–drug, drug–drug and disease–drug interactions. Pharmacogenomics. 2018;19(14):1133–8.
95. Li Y, Tang P, Cai S, Peng J, Hua G. Organoid based personalized medicine: from bench to bedside. Cell Regen. 2020;9:1–33.
96. Hennig A, Baenke F, Klimova A, Drukewitz S, Jahnke B, Brückmann S, et al. Detecting drug resistance in pancreatic cancer organoids guides optimized chemotherapy treatment. J Pathol. 2022;257(5):607–19.
97. Zushin PJH, Mukherjee S, Wu JC. FDA Modernization Act 2.0: transitioning beyond animal models with human cells, organoids, and AI/ML-based approaches. J Clin Investig. 2023. https://doi.org/10.1172/JCI175824.
98. Rudibaugh TP, Tam RW, Estridge RC, Stuppy SR, Keung AJ. Single-cell assessment of human stem cell-derived mesolimbic models and their responses to substances of abuse. Organoids. 2024;3(2):126–47.
99. Skottvoll FS, Hansen FA, Harrison S, Boger IS, Mrsa A, Restan MS, et al. Electromembrane extraction and mass spectrometry for liver organoid drug metabolism studies. Anal Chem. 2021;93(7):3576–85.
100. Ito I, Jacob S, Suzuki S, Kobayashi M, Suzuki F. Stem cell self-renewal and recovery of intestinal epithelial barrier dysfunction associated with alcohol abuse. Trends Res. 2019;2:1–11.
101. Arzua T, Yan Y, Jiang C, Logan S, Allison RL, Wells C, et al. Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell-derived three-dimensional cerebral organoids. Transl Psychiatry. 2020;10(1):347.
102. Caipa Garcia AL, Kucab JE, Al-Serori H, Beck RS, Fischer F, Hufnagel M, et al. Metabolic activation of benzo [a] pyrene by human tissue organoid cultures. Int J Mol Sci. 2022;24(1):606.
103. Apte U, Krishnamurthy P. Detoxification functions of the liver. In: Monga SPS, editor. Molecular Pathology of Liver Diseases. Boston, MA: Springer US; 2011. p. 147–63. https://doi.org/10.1007/978-1-4419-7107-4_11.
104. Tutty MA, Movia D, Prina-Mello A. Three-dimensional (3D) liver cell models-a tool for bridging the gap between animal studies and clinical trials when screening liver accumulation and toxicity of nanobiomaterials. Drug Del Trans Res. 2022;12(9):2048–74.
105. Shen JX, Youhanna S, Zandi Shafagh R, Kele J, Lauschke VM. Organotypic and microphysiological models of liver, gut, and kidney for studies of drug metabolism, pharmacokinetics, and toxicity. Chem Res Toxicol. 2019;33(1):38–60.
106. Forsythe SD, Devarasetty M, Shupe T, Bishop C, Atala A, Soker S, et al. Environmental toxin screening using human-derived 3D bioengineered liver and cardiac organoids. Front Public Health. 2018;6:103.
107. Palazzolo S, Caligiuri I, Sfriso AA, Mauceri M, Rotondo R, Campagnol D, et al. Early warnings by liver organoids on short-and long-chain PFAS toxicity. Toxics. 2022;10(2):91.
108. Tong Y, Ueyama-Toba Y, Yokota J, Matsui H, Kanai M, Mizuguchi H. Efficient hepatocyte differentiation of primary human hepatocyte-derived organoids using three dimensional nanofibers (HYDROX) and their possible application in hepatotoxicity research. Sci Rep. 2024;14(1):10846.
109. Liang S, Luo Y, Su Y, Zhang D, Wang S-j, Xu M, et al. Distinct toxicity of microplastics/TBBPA co-exposure to bioprinted liver organoids derived from hiPSCs of healthy and patient donors. Int J Bioprinting. 2024;10:1403.
110. Cheng W, Zhou Y, Xie Y, Li Y, Zhou R, Wang H, et al. Combined effect of polystyrene microplastics and bisphenol A on the human embryonic stem cells-derived liver organoids: The hepatotoxicity and lipid accumulation. Sci Total Environ. 2023;854: 158585.
111. Cheng W, Zhou Y, Chen H, Wu Q, Li Y, Wang H, et al. The iron matters: Aged microplastics disrupted the iron homeostasis in the liver organoids. Sci Total Environ. 2024;906: 167529.
112. Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, et al. Drug-induced liver injury. Nat Rev Dis Primers. 2019;5(1):58.
113. Mekky G, Seeds M, Diab AEA, Shehata AM, Ahmed-Farid OAH, Alzebdeh D, et al. The potential toxic effects of magnesium oxide nanoparticles and valproate on liver tissue. J Biochem Mol Toxicol. 2021;35(3): e22676.
114. Wei H, Li AP. Permeabilized cryopreserved human hepatocytes as an exogenous metabolic system in a novel metabolism-dependent cytotoxicity assay for the evaluation of metabolic activation and detoxification of drugs associated with drug-induced liver injuries: Results with acetaminophen, amiodarone, cyclophosphamide, ketoconazole, nefazodone, and troglitazone. Drug Metab Dispos. 2022;50(2):140–9.
115. Wu X, Jiang D, Yang Y, Li S, Ding Q. Modeling drug-induced liver injury and screening for anti-hepatofibrotic compounds using human PSC-derived organoids. Cell Regen. 2023;12(1):6.
116. Mun SJ, Ryu J-S, Lee M-O, Son YS, Oh SJ, Cho H-S, et al. Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. J Hepatol. 2019;71(5):970–85.
117. Bronsard J, Savary C, Massart J, Viel R, Moutaux L, Catheline D, et al. 3D multi-cell-type liver organoids: A new model of non-alcoholic fatty liver disease for drug safety assessments. Toxicol In Vitro. 2024;94: 105728.
118. Chen Z, Long L, Wang J, Li W, Wang A, Kankala RK, et al. Constructing tumor organoid-like tissue for reliable drug screening using liver-decellularized extracellular matrix scaffolds. ACS Omega. 2024;9(5):5888–98.
119. Almeqdadi M, Mana MD, Roper J, Yilmaz ÖH. Gut organoids: mini-tissues in culture to study intestinal physiology and disease. Am J Physiol Cell Physiol. 2019;317(3):C405–19.
120. Wang Z, Chen S, Pang Y, Ye L, Zhang Q, Jiang X, et al. Morphological alterations in C57BL/6 mouse intestinal organoids as a tool for predicting chemical-induced toxicity. Arch Toxicol. 2023;97(4):1133–46.
121. Cai T, Qi Y, Jergens A, Wannemuehler M, Barrett TA, Wang Q. Effects of six common dietary nutrients on murine intestinal organoid growth. PLoS ONE. 2018;13(2): e0191517.

122. van der Wielen N, Ten Klooster JP, Muckenschnabl S, Pieters R, Hendriks HF, Witkamp RF, et al. The noncaloric sweetener rebaudioside a stimulates glucagon-like peptide 1 release and increases enteroendocrine cell numbers in 2-dimensional mouse organoids derived from different locations of the intestine. J Nutr. 2016;146(12):2429–35.
123. Lu R, Voigt RM, Zhang Y, Kato I, Xia Y, Forsyth CB, et al. Alcohol injury damages intestinal stem cells. Alcoholism: Clin Exp Res. 2017;41(4):727–34.
124. Reding B, Carter P, Qi Y, Li Z, Wu Y, Wannemuehler M, et al. Manipulate intestinal organoids with niobium carbide nanosheets. J Biomed Mater Res, Part A. 2021;109(4):479–87.
125. Malaisé Y, Evariste L, Pettes-Duler A, Casale E, Cartier C, Gaultier E, et al., editors. Validating intestinal effects of food-grade titanium dioxide using a murine gut organoid model as alternative to in vivo models. The XVIth International Congress of Toxicology; 2022.
126. Grabinger T, Luks L, Kostadinova F, Zimberlin C, Medema JP, Leist M, et al. Ex vivo culture of intestinal crypt organoids as a model system for assessing cell death induction in intestinal epithelial cells and enteropathy. Cell death Dis. 2014;5(5):e1228.
127. Yu L, Tian X, Gao D, Lang Y, Zhang X-X, Yang C, et al. Oral administration of hydroxylated-graphene quantum dots induces intestinal injury accompanying the loss of intestinal stem cells and proliferative progenitor cells. Nanotoxicology. 2019;13(10):1409–21.
128. Flora AD, Teel LD, Smith MA, Sinclair JF, Melton-Celsa AR, O'Brien AD. Ricin crosses polarized human intestinal cells and intestines of ricin-gavaged mice without evident damage and then disseminates to mouse kidneys. PLoS ONE. 2013;8(7): e69706.
129. Garcia TM, van Roest M, Vermeulen JL, Meisner S, Smit WL, Silva J, et al. Early life antibiotics influence in vivo and in vitro mouse intestinal epithelium maturation and functioning. Cell Mol Gastroenterol Hepatol. 2021;12(3):943–81.
130. Takahashi Y, Noguchi M, Inoue Y, Sato S, Shimizu M, Kojima H, et al. Organoid-derived intestinal epithelial cells are a suitable model for preclinical toxicology and pharmacokinetic studies. IScience. 2022;25(7):104542.
131. Zhu Z, Schnell L, Müller B, Müller M, Papatheodorou P, Barth H. The antibiotic bacitracin protects human intestinal epithelial cells and stem cell-derived intestinal organoids from Clostridium difficile toxin TcdB. Stem Cells Int. 2019;2019(1):4149762.
132. Krüger J, Groß R, Conzelmann C, Müller JA, Koepke L, Sparrer KM, et al. Drug inhibition of SARS-CoV-2 replication in human pluripotent stem cell–derived intestinal organoids. Cell Mol Gastroenterol Hepatol. 2021;11(4):935–48.
133. Boj SF, Vonk AM, Statia M, Su J, Dekkers JF, Vries RR, et al. Forskolin-induced swelling in intestinal organoids: an in vitro assay for assessing drug response in cystic fibrosis patients. J Vis Exp: JoVE. 2017;120:55159.
134. Masmoudi F, Santos-Ferreira N, Pajkrt D, Wolthers KC, DeGroot J, Vlaming ML, et al. Evaluation of 3D human intestinal organoids as a platform for EV-A71 antiviral drug discovery. Cells. 2023;12(8):1138.
135. Lehmann R, Lee CM, Shugart EC, Benedetti M, Charo RA, Gartner Z, et al. Human organoids: a new dimension in cell biology. Mol Biol Cell. 2019;30(10):1129–37.
136. Xinaris C. Organoids for replacement therapy: expectations, limitations and reality. Curr Opin Organ Transplant. 2019;24(5):555–61.
137. Matthys OB, Silva AC, McDevitt TC. Engineering human organoid development ex vivo—challenges and opportunities. Curr Opin Biomed Eng. 2020;13:160–7.
138. Tidwell TR. 3D in vitro cancer models for drug screening: A study of glucose metabolism and drug response in 2D and 3D culture models: University of Stavanger; 2022.
139. Zhang S, Wan Z, Kamm RD. Vascularized organoids on a chip: strategies for engineering organoids with functional vasculature. Lab Chip. 2021;21(3):473–88.
140. Mattei G, Magliaro C, Giusti S, Ramachandran SD, Heinz S, Braspenning J, et al. On the adhesion-cohesion balance and oxygen consumption characteristics of liver organoids. PLoS ONE. 2017;12(3): e0173206.
141. Drabbe E, Pelaez D, Agarwal A. Retinal organoid chip: engineering a physiomimetic oxygen gradient for optimizing long term culture of human retinal organoids. Lab Chip. 2025. https://doi.org/10.1039/D4LC00771A.
142. Hewawasam SP. Hypoxia and oxidative stress: the role of the anaerobic gut, the hepatic arterial buffer response and other defence mechanisms of the liver. World J Meta-Anal. 2020;8(2):78–88.
143. Tse HM, Gardner G, Dominguez-Bendala J, Fraker CAJ. The importance of proper oxygenation in 3D culture. Front Bioeng Biotech. 2021;9: 634403.
144. Di Mattia M, Mauro A, Citeroni MR, Dufrusine B, Peserico A, Russo V, et al. Insight into hypoxia stemness control. Cells. 2021;10(8):2161.
145. Bouquerel C. Hypoxia-on-chip: from technological developments to biological applications: Université Paris Sciences et Lettres; 2023.
146. Polak M, Karbowniczek JE, Stachewicz U. Strategies in electrospun polymer and hybrid scaffolds for enhanced cell integration and vascularization for bone tissue engineering and organoids. Wiley Interdiscip Rev: Nanomed Nanobiotech. 2024;16(6): e2022.
147. Ameziane K. A hypoxia-maintaining perfusion device for prolonged cell studies: Tampere University; 2024.
148. Akarapipad P, Kaarj K, Liang Y, Yoon J-Y. Environmental toxicology assays using organ-on-chip. Annu Rev Anal Chem. 2021;14(1):155–83.
149. Vashishat A, Patel P, Das Gupta G, Das KB. Alternatives of animal models for biomedical research: a comprehensive review of modern approaches. Stem Cell Rev Rep. 2024;20(4):881–99.
150. Panoutsopoulos AA. Organoids, assembloids, and novel biotechnology: Steps forward in developmental and disease-related neuroscience. Neuroscientist. 2021;27(5):463–72.
151. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Sci China Chem. 2010;328(5986):1662–8.
152. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Sci China Chem. 2014;345(6194):1247125.
153. Ingber DE. Human organs-on-chips for disease modelling, drug development and personalized medicine. Nat Rev Genet. 2022;23(8):467–91.
154. Baptista LS, Porrini C, Kronemberger GS, Kelly DJ, Perrault CM. 3D organ-on-a-chip: The convergence of microphysiological systems and organoids. Front Cell Dev Biol. 2022;10:1043117.
155. Jy S, Davis RP, Mummery CL, Krauss S. Global Literature Analysis of Organoid and Organ-on-Chip Research. Adv Healthc Mater. 2024;13(21):2301067.
156. Zhang B, Radisic M. Organ-on-a-chip devices advance to market. Lab Chip. 2017;17(14):2395–420.
157. Velasco V, Shariati SA, Esfandyarpour R. Microtechnology-based methods for organoid models. Microsyst Nanoeng. 2020;6(1):76.
158. Mierke CT. Bioprinting of Cells, Organoids and Organs-on-a-Chip Together with Hydrogels Improves Structural and Mechanical Cues. Cells. 2024;13(19):1638.
159. Low LA, Tagle DA. Organs-on-chips: Progress, challenges, and future directions. Exp Biol Med. 2017;242(16):1573–8.
160. Lampart FL, Iber D, Doumpas N. Organoids in high-throughput and high-content screenings. Front Chem Eng. 2023;5:1120348.
161. Zhao Y, Landau S, Okhovatian S, Liu C, Lu RXZ, Lai BFL, et al. Integrating organoids and organ-on-a-chip devices. Nat Rev Bioeng. 2024;2(7):588–608.
162. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol. 2014;32(8):760–72.
163. Zhu Y, Sun L, Wu X, Gu Z, Zhao Y. Engineered Human Organoids for Biomedical Applications. Adv Func Mater. 2024;34(11):2310961.
164. Goldrick C, Guri I, Herrera-Oropeza G, O'Brien-Gore C, Roy E, Wojtynska M, et al. 3D multicellular systems in disease modelling: From organoids to organ-on-chip. Front Cell Dev Biol. 2023;11:1083175.
165. Laufer MC. Organ-on-a-chip technologies to study neuromuscular disorders: possibilities, limitations, and future hopes. Med Gen. 2021;33(3):261–7.
166. Corrò C, Novellasdemunt L, Li VS. A brief history of organoids. Am J Physiol Cell Physiol. 2020;319(1):C151–65.
167. Feitor JF, Brazaca LC, Lima AM, Ferreira VG, Kassab G, Bagnato VS, et al. Organ-on-a-Chip for drug screening: a bright future for sustainability? A critical review. ACS Biomater Sci Eng. 2023;9(5):2220–34.
168. Jie M, Lin H, He Z, Liu H, Li H, Lin J-M. An on-chip intestine-liver model for multiple drugs absorption and metabolism behavior simulation. Sci China Chem. 2018;61:236–42.

Publisher's Note