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Reviews

Emulating the gut–liver axis: Dissecting the microbiome's effect on drug metabolism using multiorgan-on-chip models

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Abstract

The homeostatic relationship between the gut, its microbiome, and the liver is crucial for the regulation of drug metabolism processes. Gut microbes are known to influence human health and disease by enhancing food metabolism and providing a first line of defense against pathogens. In addition to this, the gut microbiome also plays a key role in the processing of exogenous pharmaceutical compounds. Modeling the highly variable luminal gut environment and understanding how gut microbes can modulate drug availability or induce liver toxicity remains a challenge. However, microfluidics-based technologies such as organ-on-chips could overcome current challenges in drug toxicity assessment assays because these technologies are able to better recapitulate complex human responses. Efforts are being made to create *in vitro* multiorgan platforms, tailored for an individual patient's microbial background. These platforms could be used as a tool to predict the effect of the gut microbiome on pharmacokinetics in a personalized way.

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Gut–liver axis, Gut microbiome, Organ-on-chip, Microfluidics, Drug metabolism, Liver metabolism.

Introduction

Gut microbiome and gut-derived metabolites play key roles in biotransformation of drugs, by directly or indirectly influencing drug absorption, toxicity, and bioavailability [1]. Previous work on the gut–liver axis has shown that the interplay between the intestinal microbiome, the gut barrier, and the liver is fundamental for regulating drug metabolism processes [2]. Disruption of these processes, caused by dysbiosis, alcohol consumption, administration of antibiotics, or genetic susceptibility may lead to gut barrier dysfunction. As a result, this causes uncontrolled translocation of bacteria and their metabolites into the liver via the portal vein [3]. In contrast, the use of probiotics can enable reconstitution of microbial homeostasis and improve drug efficacy [4]. The human microbiome is constantly subject to transformations (nutritional status, antibiotics, and pathology) and is therefore highly variable [5–7]. Hence, interindividual and intraindividual variation in drug response must be taken into consideration when assessing drug safety and toxicity. Key metabolic processes can be studied using *in vitro* and *in vivo* models, that is, transwell systems and rodent models [8,9]. However, these models do not fully recapitulate the human gastrointestinal physiology [10]. Although animal models enable *in vivo* analysis, the human physiological mechanisms and complexity make it difficult to assess human-specific drug responses. Therefore, many drugs do not show adverse effects on animals but lead to unexpected impairments in humans during clinical trials [9]. In addition, substantial differences in expression levels of drug-metabolizing enzymes have been found between the intestine and the liver of rodents and humans [11]. Hence, there is a need to develop new tools to model the gut–liver cross talk. Organ-on-chips (OoCs) have the potential to better recapitulate organ physiology *in vitro* and offer reliable and precise control of cellular, biochemical, and biophysical parameters [12,13]. These microfluidics-based technologies allow even high-throughput testing and thus represent attractive and resource-efficient alternatives to animal experimentation [9]. OoCs with differentiated human-induced pluripotent stem cells (hiPSCs) offer new possibilities to provide a path to a more personalized

medicine by recreating a patient-specific organotypic microenvironment for drug testing [14,15]. Thus, these chips have gained increased interest and have the potential to revolutionize processes in drug development and testing.

Here, we outline the importance of the intestinal microbiome in modulating drug metabolism. We also highlight how novel microfluidics-based tissue models could provide novel insights into the signaling along the gut–liver axis and help to advance our understanding in drug-induced toxicity and its underlying mechanisms. Finally, we discuss how the combination of microfluidic/OoC technologies and personalized medicine approaches could lead to a paradigm shift of the current drug development practice.

Importance of gut microbiome homeostasis and gut barrier function

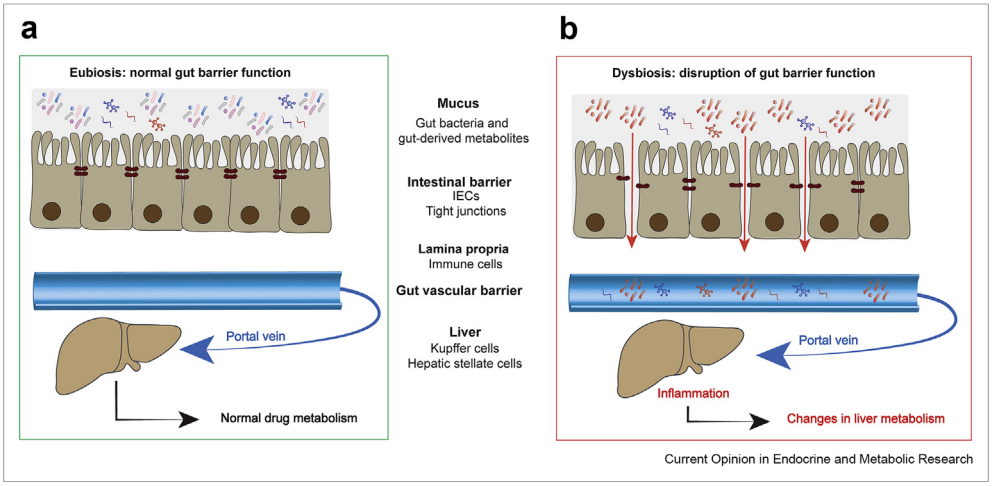
The gastrointestinal tract harbors trillions of microorganisms, also known as the gut microbiome [16]. The microbial composition is a key element in regulating food digestion, shaping the immune system, and maintaining the gut barrier [17]. Under eubiotic conditions, the gut microbiome is characterized by a predominance of beneficial bacterial species, that is, belonging to the two bacterial phylum Firmicutes and Bacteroides [16]. When there is an optimal balance in the gut microbiome composition, drug metabolism processes are tightly regulated [6,18] (Figure 1a). In contrast, under dysbiotic conditions, that is, when potentially pathogenic bacterial species belonging to the phylum Proteobacteria may be predominant, drug metabolism processes are compromised [6,16,18] (Figure 1b). Disruption of the gut barrier due to deregulation of tight junction complexes causes elevated translocation of microbes and microbe-associated molecular patterns (MAMPs) [19,20]. The uncontrolled dissemination of microorganisms and MAMPs into circulation activates inflammatory signaling pathways by activating Toll-like receptors expressed by liver cells, that is, Kupffer cells and hepatic stellate cells. The induction of these signaling pathways contributes to aggravation of liver inflammation and modifies drug metabolism by hepatocytes [21]. The consequences of intestinal dysbiosis and a disrupted gut barrier function are further demonstrated by development of chronic liver diseases, for example, alcoholic liver disease and nonalcoholic fatty liver disease [22,23]. Both diseases are characterized by an increased translocation of microbes and MAMPs into circulation, eventually reaching the liver and triggering the onset of local inflammatory signaling [24]. The induction of proinflammatory pathways subsequently causes liver fibrosis and cirrhosis that could ultimately give rise to formation of hepatocellular carcinoma.

Roles of the gut microbiome and the liver in drug metabolism

Importance of the gut–liver axis in drug metabolism

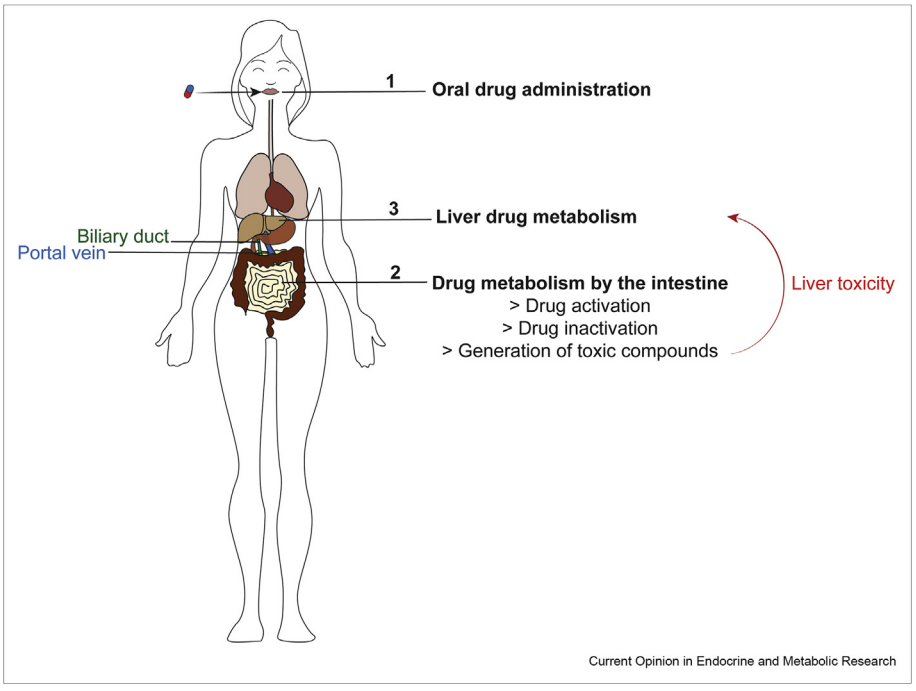
Pharmaceutical compounds administered via the oral route are metabolized by organs of the gastrointestinal tract (Figure 2). Given that there is a bidirectional relationship between the liver and the intestine, the portal vein ensures the passage of drug metabolites from the intestine to the liver, and some drugs or drug metabolites are secreted back to the intestine via the biliary duct [25]. This process known as enterohepatic

Figure 1



Drug metabolism under healthy and dysbiotic conditions. (a) Gut barrier under healthy (eubiotic) conditions. Under eubiotic conditions, the infiltration of gut microbes and the dissemination of related metabolites and toxins through the intestinal wall are tightly controlled through the presence of a mucous layer and a tight intestinal barrier. Under such homeostatic conditions, ingested drugs are normally metabolized. **(b) Gut barrier under dysbiotic conditions**. A dysbiotic state is characterized by the disruption of tight junction complexes and IECs. Subsequently, gut bacteria and gut-derived metabolites are translocated into the liver via the portal vein, causing inflammation and inducing changes in liver metabolism processes. IECs, intestinal epithelial cells.

Figure 2



Importance of the gut microbiome in drug metabolism. After oral administration (1) of a pharmaceutical drug, the product is absorbed in the small intestine, where it is being metabolized by host and microbial enzymes (2). At this stage, drugs can be either activated, be inactivated, or generate toxic compounds. Then, the metabolic product enters the liver via the portal vein (blue), where it is being further metabolized (3). Finally, some drugs or drug metabolites are excreted into the bile and return to the intestine via the biliary tract (green). The passage of toxic compounds from the intestine to the liver can lead to liver toxicity.

circulation can result in reactivation of the drug by commensal bacteria residing in the intestine. Given that the drug is not properly detoxified and its circulation in the body is prolonged, this may result in liver toxicity [11]. The gut is an important factor in regulating drug metabolism process as it expresses host and microbe-derived enzymes, capable of metabolic reactions.

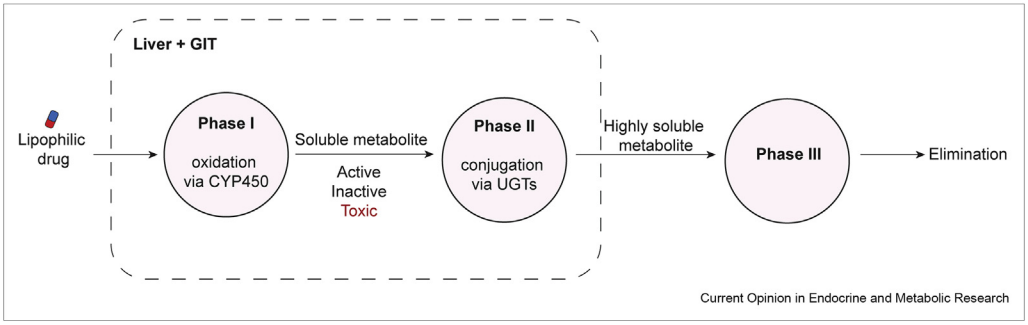
Drug metabolism involves chemical biotransformation of drug molecules by enzymes expressed either by the host or by gut microbes [6,18]. Metabolic processes differ along the intestinal tract and also among different species. These differences can be explained by the presence of different isoforms of drug-metabolizing enzymes between two different species, for example, cytochrome P450 (CYP450) enzymes and by variances in expression profiles of these CYP450 enzymes along the intestinal tract within the same species [11]. For instance, levels of CYP450 enzymes diminish along the intestinal tract and are thus lowest in the colon. Drug absorption in the intestine is also highly influenced by variations in the luminal gut environment, for example, pH and expression of transporters. The microbial composition and abundance differ along the intestinal tract, which also account for differences in metabolism [26]. For instance, the upper small intestine has lower levels of bacteria, which are mostly *Streptococci* and *Lactobacilli*, whereas the colon has increased levels of bacteria, which are mainly anaerobic.

The main purpose of drug metabolism is the formation of more hydrophilic compounds to facilitate the elimination of drugs and their metabolites [27] (Figure 3). On the one hand, some metabolites are required for drug action (in the case of prodrug administration), and on the other hand, some metabolites can lead to the generation of toxic compounds [28,29]. To avoid liver toxicity, drug development processes aim to bypass the generation of such reactive compounds.

Microbial drug metabolism and its consequences on drug metabolism

Recent studies have identified microbiome-induced changes in hepatic drug metabolism [29]. In addition, recent studies indicate that the intestinal microbiome can directly or indirectly affect drug pharmacokinetics [1,29–32]. Drugs that are delivered orally come into contact with commensal bacteria in the small and large intestine, where they are subject not only to host enzymes but also to microbial drug-metabolizing enzymes. A recent study analyzed the ability of several human gut bacteria from diverse clades to metabolize orally

Figure 3



Drug metabolism. A chemical compound that is administered orally is subjected to several metabolic pathways that can be subdivided into three phases. The liver is the main organ implicated in drug metabolism. The gastrointestinal tract (GIT) also takes part in the first two phases of drug metabolism. Phase I is characterized by oxidation reactions, which are mainly catalyzed by cytochrome P450 (CYP450) enzymes. Phase I transforms lipophilic drugs into soluble metabolites. Here, activation and inactivation of the drug can occur. In addition, toxic by-products can also be generated through this process. Phase II is known as the detoxification pathway. This phase implicates the conjugation of phase I–derived metabolites via UDP-glucuronosyltransferases (UGTs), resulting in formation of highly soluble metabolites. These hydrophilic metabolites can then be eliminated from the body during phase III.

administered drugs and reported a series of drugs that are chemically modified by gut microbes [30]. They were also able to identify microbially encoded enzymes that can impact drug metabolism.

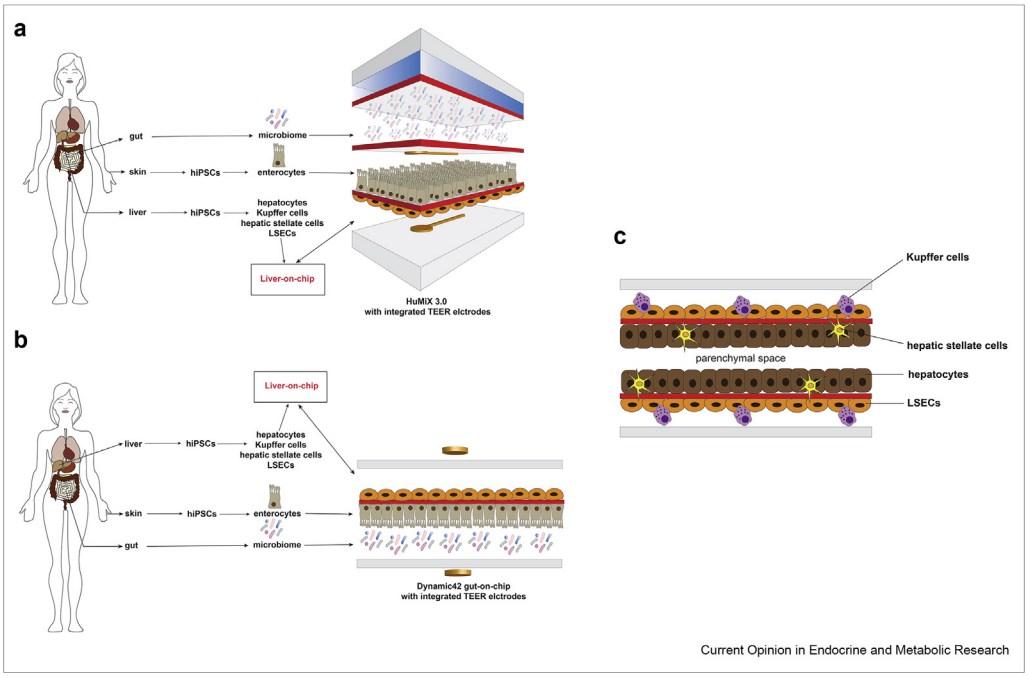
In fact, microbes have the ability to activate, inactivate, or generate toxic compounds via metabolic processes (Figure 3). For instance, digoxin, a cardiotonic drug, can be inactivated by *Eggerthella lenta* through conversion into dihydrodigoxin and dihydrodigoxigenin [33]. Levodopa (L-Dopa), used for the treatment of Parkinson disease, needs to be absorbed by the small intestine to cross the blood–brain barrier, to undergo decarboxylation within the central nervous system, and to be transformed into the therapeutically active dopamine [34]. However, it has been shown that L-Dopa is transformed into dopamine by *Enterococcus faecalis*–mediated decarboxylation, which contributes to side effects in the gastrointestinal tract [35]. Then, dopamine is further dehydroxylated to m-tyramine by *E. lenta*, which influences dopamine-associated side effects in the gut. Thus, L-dopa is often coadministered with carbidopa, a drug that inhibits peripheral metabolism. Sulfasalazine, an anti-inflammatory drug, is activated by microbial enzymes via enzymatic cleavage of an azo bond. Irinotecan, an anticancer drug, is first hydrolyzed by an enzyme to give rise to an active compound and exert its pharmacological effect [36]. Subsequently, it is subjected to conjugation via UDP-glucuronosyltransferases in the liver and rendered in a biologically inactive state. When transferred to the intestine, it is deconjugated by gut microbes, for example, *Escherichia coli*, *Fusobacterium nucleatum*, and *Clostridium ramosum*, and could mediate toxic effects in the lower gastrointestinal tract [37]. Brivudine is an oral antiviral drug metabolized by human enzymes and the gut microbiome to bromovinyluracil, which is able to induce hepatoxicity upon activation in the gut [32].

The intestinal microbiome influences xenobiotic metabolism, with the microbial composition being highly variable between individuals, resulting also in a high variability of microbial drug metabolizing-enzymes. This fact may contribute to the interindividual variability frequently observed for individual responses to drug treatments [38]. Thus, a better understanding of microbial drug metabolism is necessary to customize treatments and to further the understanding of how an individual's microbiome could be manipulated to increase drug efficacy.

Drug testing and safety assessment in OoC models

Animals are frequently used in preclinical trials to evaluate drug toxicity [9]. Although animal models enable long-term studies of pharmacokinetics in a multiorgan system, they have drawbacks. As mentioned beforehand, there are significant differences in expression levels of drug-metabolizing enzymes and transporters between the intestine and the liver of rodents and humans [11]. Furthermore, rodent microbial communities significantly differ from human microbiome in their diversity and richness [8]. Interestingly, it has recently been demonstrated that although mouse and human gut microbial communities are extensively different, there is a high similarity at the functional level, for example, conserved functions of enzymes that catalyze the breakdown and biosynthesis of carbohydrates and glycoconjugates [39]. Although rodent models can be manipulated through diet and genetics and at the microbial level to explore gut–microbiome interactions, multiple species have to be used for

Figure 4



Personalized multiorgan chips to model the gut–liver axis. hiPSCs are derived from a patient's tissue (e.g. skin) and can then be reprogrammed and later differentiated into specialized organ-specific cells (enterocytes, hepatocytes, Kupffer cells, hepatic stellate cells, and liver sinusoidal endothelial cells [LSECs]). Here, we propose to integrate major cell types with a patient's preserved genetic background and patient-derived gut microbiome into a gut–liver-on-chip system. **(a) Schematic of HuMiX including a connection to a liver-on-chip system**. HuMiX harbors four parallel channels, separated by semipermeable membranes (red). HuMiX enables the coculture of human cells (intestinal epithelial cells) and microbes (second and third layer, respectively). The presence of a nitrogen (N_2) flow in the top channel establishes an anoxic environment for culturing anaerobic bacterial species from the gut. The environment within the device is routinely monitored through oxygen (O_2) sensors. Integrated transepithelial electrical resistance (TEER) sensors (yellow) will ensure on-chip monitoring of the gut barrier integrity. The modular design of HuMiX allows downstream analysis. In the interconnected gut–liver system that we propose, the upper chamber of the Dynamic42 liver-on-chip platform would be connected to the lower chamber of HuMiX, harboring the iPSC-derived gut endothelial cells. **(b) Schematic of the Dynamic42 gut-on-chip platform with a connection to a liver-on-chip system**. The Dynamic42 gut-on-chip platform will contain hiPSC-derived epithelial and endothelial cells and offers the possibility for the integration of patient-derived gut microbes. Moreover, TEER electrodes will be integrated to monitor the gut barrier integrity. **(c) Schematic of the liver-on-chip system including hiPSC-derived cell types**. The Dynamic42 liver-on-chip platform enables the integration of hiPSC-derived LSECs and Kupffer cells in the upper chamber. hiPSC-derived hepatocytes and hepatic stellate cells will be integrated in the lower chamber. Both of these chambers are separated by a porous membrane. hiPSCs, human-induced pluripotent stem cells.

animal testing, and its results must be combined to predict drug effects in humans. Thus, alternative testing systems are urgently required to emulate human physiology of the respective organ *in vitro* and to better recapitulate the essential aspects of human metabolizing pathways. OoCs, also known as microphysiological systems, are able to recapitulate the 3D structure and cellular diversity of human tissues and the biochemical and biophysical cues exposed to these tissues at various levels [12,13,15,40–42].

Advanced cell sources such as hiPSCs become frequently used in novel *in vitro* models because they have their ability to differentiate *in vitro* into virtually any cell type [43]. In addition, hiPSCs possess an improved self-renewal capacity and thus provide an unlimited cell source for generation of tissues and organoids. Current genetic editing methods leverage the generation of isogenic controls and address more specific mechanistic questions. Thus, hiPSCs provide a powerful tool to generate major organ-specific cell types reflecting the patient's genetic background. It has been recently demonstrated that stem cell–derived organoids can be efficiently cultured in OoC platforms, thereby increasing their cellular diversity and supporting the differentiation of even rare cell types by extending the organoid's life span and its cell mass [44].

Multiorgan models

OoC platforms further allow the generation of multiorgan models to study drug metabolism and related effects along the gut–liver axis. For instance, a microfluidics-based system coupling two microchambers containing intestinal and liver tissue has been

developed and used to investigate interorgan communication. More recently, a multiorgan gut–liver model has been developed by interconnecting single-OoC systems [45]. Furthermore, a four-organ chip has been established for *in vitro* ADME (absorption, distribution, metabolism, and elimination) profiling and drug toxicity testing [46].

Here, we present two options for more advanced models by combining the previously validated OoC platforms HuMiX [47–49] (Figure 4a) and the Dynamic42 Biochip platform [50,51] (Figure 4b and c). The human–microbial cross talk model (HuMiX) has been validated as an effective model to recreate the complex structure and physiology of the intestinal epithelium [47]. Importantly, recent findings have shown that HuMiX-based readouts are concordant with *in vivo* data [49]. Hence, HuMiX provides a tool for *in vitro* drug testing and for the study of the effects of the intestinal microbiome on drug availability. A 3D gut-on-chip model has also been established on the Dynamic42 biochip platform to investigate microbiome–host interaction in an immunocompetent microenvironment [50] (Figure 4b). The same platform was used to establish a liver-on-chip model reflecting essential aspects of liver metabolism and microanatomical features of the human liver sinusoid [51,52] (Figure 4c). The system has been proofed as a suitable platform for drug metabolism studies, in the development of novel drug delivery systems and in disease modeling studies of acute and chronic liver inflammation [53–55]. We propose to interface both OoC platforms to emulate metabolization and drug metabolism along the gut–liver axis *in vitro* (Figure 4a and b). Transepithelial electrical resistance electrodes capable of measuring the formation and disruption of epithelial monolayers in real time and in a noninvasive way will be integrated in both platforms.

Linking OoCs involves consideration of biological parameters, for example, sterility, universal medium, flow rates, and a common source of cells, to create a physiologically relevant and functional multi-OoC model [12]. Thus, we first plan to assess the viability and the metabolic profiling of the cells in the established platforms and study phenotypical and functional metrics for normal gut and liver function in comparison with a disease model. Second, we aim to validate the platforms by tackling a specific research question related to compounds of known action or toxicity.

Future perspectives

Major challenges in establishing an appropriate clinical model for drug metabolism need to be considered. These include drug transit through the gastrointestinal tract and the liver, drug permeability and absorption, drug solubility, intestinal blood flow, and expression of drug-metabolizing enzymes and transporters. OoCs are being developed to model more simplified physiological parameters and to mimic only parts of an organ to find the right balance between complexity and feasibility. Despite this fact, their design enables precise control of cell culture parameters, the possibility of coculture, and the application of different flow rates to each compartment to match physiological parameters to create a simple model that recapitulates physiological responses of interest.

An important advantage of OoC platforms consists in their versatile application to characterize drug metabolization and mechanisms of drug-mediated toxicity at different molecular levels by use of scalable tissue models. Cell sources and cellular diversity of the tissues and organoids can be easily adapted to precisely dissect individual contributing factors on a standardized platform offering a multitude of functional readouts. With the ability to adjust the cellular complexity level ranging from simpler but highly robust 2D cell cultures up to complex stem cell–based 3D organoid models cultured under precisely regulated conditions, the OoC platform provides superior options in their application in drug metabolization studies. Microfluidic perfusion further allows the integration of peripheral immune cells to further expand such studies, that is, for assessment of immunomodulating therapies including chimeric antigen receptor T cells and bivalent antibodies to fight cancer. Recent data indicate an important role of the microbiome in the effectiveness and safety of these therapies [56]. OoC models allow a structured approach to dissect individual contributing factors for both the host and the microbiome by modulating the host genetic background (i.e. use of clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) tools) on one side and iterative testing of individual factors at the microbiome level by use of suspended MAMPs and stool sample filtrates up to defined microbial communities and isolated complex human microbiome samples derived from patients and healthy donors.

The establishment of a unifying platform to integrate different organ models already established and characterized in a plug-and-play manner will further increase potential of OoC in studies addressing the systemic level of organ–organ communication. We here propose the combination of gut- and liver-on-chip models developed by our groups to recreate the gut–liver axis. The generation of hiPSC-based gut and liver tissue from the same donor cell line will ultimately allow the generation of physiologically relevant models, avoiding allogenic cytotoxicity by mixing of cells from different donors. Considering appropriate cell numbers, tissue size, and cell/volume will be key to reliably emulate physiologically relevant multiorgan systems [57]. Further examples of such models include the gut–lung and the gut–brain axis. Similar to the liver axis, the

influence of the gut microbiome on the function of the human lung and central nervous system is well established, and dysbiotic conditions of the gut have been proven to be associated with the onset of respiratory diseases of the lung and mental disorders of the brain [58,59]. The improvement of current OoC platforms by implementation of high-throughput screening capabilities and computational analysis tools will allow the development of next-generation *in vitro* models. Thus, we are convinced that next-generation multi-OoC models will represent a powerful alternative to stepwise replacement of the use of animal experimentation with regard to the microbiome and its role in drug metabolization for individual patients.

Conflict of interest statement

Nothing declared.

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