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IUPHAR Invited Review

The application of organ-on-chip models for the prediction of human pharmacokinetic profiles during drug development

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ABSTRACT

Organ-on-chip (OoC) technology has led to *in vitro* models with many new possibilities compared to conventional *in vitro* and *in vivo* models. In this review, the potential of OoC models to improve the prediction of human oral bioavailability and intrinsic clearance is discussed, with a focus on the functionality of the models and the application in current drug development practice. Multi-OoC models demonstrating the application for pharmacokinetic (PK) studies are summarized and existing challenges are identified. Physiological parameters for a minimal viable platform of a multi-OoC model to study PK are provided, together with PK specific read-outs and recommendations for relevant reference compounds to validate the model. Finally, the translation to *in vivo* PK profiles is discussed, which will be required to routinely apply OoC models during drug development.

1. Introduction

The development of novel drugs is a lengthy, labor-intensive and costly process. Before a drug candidate can enter the clinical phase, regulatory agencies require pre-clinical data on efficacy, toxicity and pharmacokinetics (PK). The latter is defined by absorption, distribution, metabolism and excretion (ADME) of a drug candidate, which together determine the amount of compound reaching target sites, and thereby the efficacy and potential off-target toxicity. Although there is an elaborate preclinical screening and characterization of compounds along with a large amount of resources invested in this process, almost 90% of drug candidates that enter phase I clinical trials will ultimately fail because they lack efficacy or due to unforeseen side effects [1,2].

The preferred route of systemic drug delivery is *via* oral administration, with a particular ease of use for the patient, a high willingness to take medication and the lowest costs for treatment. However, not all compounds administered orally will reach their target site(s). Oral bioavailability (F) refers to the extent of a substance or drug that becomes available to the systemic circulation [3,4], which is in addition to release from the formulation, determined by the absorption of the drug at the intestine (F_a), first-pass metabolism in the intestine (F_g) and/or liver (F_h), and enterohepatic circulation. Hepatic (CL_h) and renal (CL_r)

Abbreviations: ADME, absorption, distribution, disposition and excretion; ALDH, aldehyde dehydrogenase; ASPGR, asialoglycoprotein receptor; AOX, aldehyde oxidase; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CES, carboxylesterase; CL_h, hepatic clearance; CL_r, renal clearance; CYP, cytochrome P450; DDI, drug-drug interactions; F, oral bioavailability; F_a, fraction absorbed; FDA, Food and Drug Administration; F_e, fraction excreted unchanged in urine by the kidney; F_g, fraction escaping metabolism in the intestine; F_h, fraction escaping metabolism in the liver; GST, glutathione S-transferase; MATE, multidrug and toxic compound extrusion; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; OoC, Organ-on-chip; P-gp, p-glycoprotein; PDMS, polydimethylsiloxane; PK, Pharmacokinetics; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

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clearance, subsequently, contribute to drug elimination from the systemic circulation. To make the drug development process more efficient and less expensive, it is key to predict the oral bioavailability of a new compound accurately to select compounds that achieve adequate systemic exposure.

Traditionally, animal models and conventional 2D cell culture models are used in the preclinical phases of drug development. Despite many high impact and cross-referenced publications, PK research using rodents, dogs and non-human primates has variable translational success and, thus, has often limited predictive value for human PK [5–7]. Besides, the rising ethical constraints and high costs involved in using these models increase the demand for alternative *in vitro* models. Currently, the conventional cell culture models still fail to mimic the complexity of *in vivo* human tissue [8]. Organ-on-chip is a rapidly emerging *in vitro* technique that – at least partially – can omit the issues with animal models and conventional cell culture models. In this review, the potential of organ-on-chip models to improve the prediction of human oral bioavailability and intrinsic clearance is discussed. Specifically, the focus will be on the functionality of the models rather than the technical aspects, and the application of organ-on-chip technology in current drug development practice.

2. Organ-on-chip models

Organ-on-chip (OoC) is a cell culture technique that aims at replicating the physiological characteristics of organs *in vivo*, by reconstructing the structure and function *in vitro* [9,10]. The devices are typically made using microfabrication methods such as soft lithography, micro-machining and injection molding, or by 3D printing. They are designed to house specific organotypic tissues or cells in a 3D environment with networks of channels. Despite the large variety of different systems, there are several common features of organ-on-chip models: (1) they include cells or tissue of human origin, (2) there is flow to allow nutrient and gas exchange, and waste removal (3) environmental control of (bio)mechanical or electrical stimulation, and (4) they allow drug dosing and biological read-outs. The practical advantages of OoC models compared to conventional 2D cell cultures are reviewed elsewhere [10–12]. In short, the main drawback of conventional cell culture is the loss of tissue-specific functions, such as accurate barrier formation or metabolic capacity. OoC models provide optimized nutrient and oxygen supply to the cells, together with a 3D growth architecture and potential interactions between multiple different cell types. In addition, the medium flow in OoC has a significant impact on cellular metabolism, providing biological relevant shear stress to the cells and preventing accumulation of (toxic) metabolites in the culture medium. Together, this results in an improved replication of human biology and physiology. Experimental data gathered in OoC systems can then be extrapolated to transport and metabolism properties of tissues *in vivo* by mathematical modelling [13,14].

The recent developments in OoC technology have led to *in vitro* techniques with new opportunities for ADME/PK studies:

- Study human biological variation in response to compounds. In some clinical trials, there are significant differences in responses to therapy observed among different genetic populations [15]. Advanced *in vitro* models like OoC that include cells with genetic variation have the potential to identify which subpopulation of patients is at risk, or if the optimal dose should be adjusted for specific patient groups. This potentially results in an improved clinical trial design. Furthermore, it can also enable drug candidate testing in vulnerable populations like pregnant women, children and elderly, which are often excluded from clinical trials.
- Study the effects of long-term drug exposure *in vitro*. With the improvement of culture techniques, also the longevity of the cells and tissues *in vitro* has improved. This enables the investigation of prolonged and/or repeated drug exposure and subsequent long-term effects.
- Connection of multiple organs. Due to the 3D design of the chips, with complex networks of channels, it is possible to included different tissue types in one chip. In addition, the fluid flow can even enable connection of separate organ-on-chip modules in a physiologically relevant way, building a complete body-on-chip system.
- Evaluate complex drug-drug interactions (DDIs). When age increases, the number of diseases also increase resulting in polypharmacy [16]. A drug metabolized by a specific enzyme will have a higher clearance rate (resulting in lower effectivity) if administered simultaneously with a drug that induces that specific enzyme. Investigating DDIs in animal models or conventional *in vitro* models is often limited to the interaction between two drugs. OoC models allow to study the effect of multiple drug combinations and time-dependent inhibition.
- Reduction of animal experiments. Another major advantage of the implementation of OoC in drug development is that it might lead to the reduction and replacement of animal experiments, which is in line with the 3 R principles that are embedded in legislation and regulations [17]. Very recently, the Modernization act 2.0 was signed, stating that new medicines do not strictly require animal testing anymore to receive U.S. Food and Drug Administration (FDA) approval, providing more opportunities for alternative preclinical models.
- Test novel human-specific drug candidates. Many of the most recently developed treatments for human diseases are biotherapeutics, including monoclonal antibodies, oligonucleotides, small interfering RNAs (siRNAs) and viral gene vectors. They can be so specific for human target molecular sequences that they should not show any activity in non-human models and, therefore, cannot be tested in animals. Over the past years, more than ten antisense oligonucleotide drugs [18] and five siRNAs [19] have been approved by the FDA. Following intravenous, intrathecal or subcutaneous dosing, liver and kidney are in general the organs with the highest uptake of the oligonucleotides [20]. Some oligonucleotides are conjugated with N-acetylgalactosamine (GalNAc), a high-affinity ligand for the Asialoglycoprotein receptor (ASPGR) that is highly expressed on hepatocytes, resulting in rapid endocytosis [21,22]. Inside the organs, the oligonucleotides are cleaved by nucleases. As the resulting shortmers could still be pharmacologically active, there is a need to develop suitable *in vitro* systems to study their metabolism [20]. As the uptake and metabolism are slow, *in vitro* models with long term proliferative capacity are required for this. A predictive preclinical *in vitro* OoC model that is capturing accurate liver uptake receptors, enzymes and transporters for antisense oligonucleotides can be used in the screening process of therapeutic oligonucleotides. For peptides and proteins, another class of biotherapeutics, the kidney plays an important role in their metabolism as well as in their elimination. For radioligands with peptide backbones, like Lutathera, the kidney is the main dose-limiting organ in radioligand therapy (RLT), and there is an urgent need for reducing renal radioactivity accumulation [23,24]. Thus, predictive *in vitro* OoC models demonstrating efficient elimination and clearance from the kidney would be of great benefit for RLTs.

In summary, the OoC technology has led to *in vitro* models with many new possibilities compared to conventional *in vitro* and *in vivo* models. It is estimated that the implementation of OoC technology in drug development could save up to 25% of total research and development costs per drug candidate [25].

2.1. Existing challenges

Although promising, the field of OoC is relatively new and still in development. The current limitations of OoC models are extensively

discussed elsewhere [26–29]. In summary, most models show improved human relevance compared to traditional models but are not yet able to fully reflect the key characteristics of human physiology, which impairs clinical translation. Existing challenges that need to be resolved at present:

- Non-relevant cell source. Most models include standardized cancer cell-lines that bear several mutations and have lost certain cell-specific functions. In addition, these cell lines do not represent the large biological variation present in the human population. Primary cells are used as alternative, but the loss of relevant drug transporters and metabolizing enzymes activity over time is a main issue, together with the high variability between batches [30]. Models that apply *ex vivo* tissue experience limitations in availability and viability. The latter can be improved by using tissue-derived organoids. Recently, the use and availability of iPSC-derived cells in OoC models also increased, but this technique still displays high variation in the level of differentiation and maturation of the cells [31].
- Not animal-free. OoC models claim to replace animal testing, thereby contributing to the current focus on the 3Rs (reduction, refinement and replacement). However, in many *in vitro* culture methods there are still animal-derived compounds like extracellular matrix proteins or fetal calf serum present. The development of true animal-free alternatives for these compounds deserves further attention.
- Inadequate chip materials and manufacturing properties. There is a substantial variation in materials and techniques used to produce OoC models. The technical details of designing organ chips, connection strategies for multiple organ models and medium flow strategies are reviewed elsewhere [27,32–34]. Several OoC models use polydimethylsiloxane (PDMS) for fast, flexible and relatively low-cost fabrication of chips that are biocompatible and can be used in various applications [4]. A disadvantage for drug studies is that PDMS strongly absorbs small hydrophobic molecules. However, this may be less of an issue for biologics, which are the largest number of novel drugs currently being developed. Besides materials used, the technical set-up and connection strategies are also still a challenge in multi-OoC models. Current advancements in microfluidic technology will further improve OoC models, but can also result in more complexity and increased costs of manufacturing. Robust device construction and material supply are essential for multi-OoC commercialization. An additional consideration is the application of re-usable devices instead of disposables, decreasing the environmental impact of the drug development pipeline.
- Restricted sampling. Key ADME-related readouts are liquid chromatography–mass spectrometry (LC-MS) based quantification of test compounds and metabolites, and enzyme-linked immunosorbent assays (ELISAs) for biologics and biomarkers. The design of some OoC models does not allow access to all tissue compartments, limiting the ability to sample for mechanistic investigations. In addition, the small scale of the devices results in low cell number and/or media volume available for downstream analyses. Furthermore, the material of the chip might prevent imaging as a tool for readouts.
- Lack of standardization and validation. With complex multi-OoC models, there are many components that require standardization: the cell system, the chip material, the scaffold, the medium, the microfluidic flow rate, the endpoints measured, *etc.* It needs to be established to which extent biological variation should be included to obtain reliable results, and how many chips are required to obtain valuable test results for one novel drug compound. Open platform technology will accelerate standardization of OoC models, and several new initiatives to facilitate this are emerging, *e.g.* Moore4Medical [35], SMART Organ-on-Chip [36] and NXTGEN HighTech [37]. To obtain acceptance by health authorities to replace animal studies by OoC models, a thorough model validation is also required. The extent of validation needed will depend on the *in vitro* endpoint of the model and its relevance for human safety.

To improve current models, all relevant stakeholders should be involved in the design, development and optimization of multi-OoC models: researchers, technical developers, end-users and regulatory bodies. This ensures models will be developed with both high translational value but also high chance of application in common practice and acceptance.

2.2. PK OoC model application in drug development

There are several consecutive phases in the drug developmental pipeline, from early discovery *via* pre-clinical studies to clinical studies (Fig. 1) [38]. The OoC technology is not applicable for quantitative high-throughput screening during early drug discovery. The technical complexity of most models makes them low throughput but high content. The use of OoC models, specifically those designed for PK studies, is therefore more relevant during the candidate selection phase, where they can be applied for further, mechanistic testing. The models have the potential to increase understanding of certain PK behavior of a compound, *i.e.* the interplay between absorption and metabolism, and provide an estimation of bioavailability [39]. In addition, it might reveal the distinction between hepatic and extrahepatic metabolism of a compound, and provide information on the balance between hepatic and renal excretion. Besides, OoC models can provide information on specific metabolites or biomarkers that are important to monitor during the clinical phase [40].

OoC models can provide relevant information on ADME/PK safety for an investigational new drug (IND) application, required to enter the human clinical trial phase. Connected multi-OoC models allow the investigation of inter-organ crosstalk and identification of potential side-effects [41]. The option of continuous dosing and/or sampling and the control of culture conditions, allows to test complex dosing regimens or cycles, even for combination therapies. The models also provide a relevant platform to study drug-drug-interactions (DDI) [42]. Complex DDI involving multiple organs are associated with increased uncertainty when combining data from multiple independent experiments in single tissues. Collecting information on DDI in multi-OoC before the clinical phase can prevent the unnecessary exclusion of patients on certain drug candidates from the clinical study.

For the clinical phase, OoC models can reveal populational variation in responses, thereby providing input for better design of the clinical studies [38]. Specific OoC models can recapitulate a biological process of a specific disease that is not captured in traditional cell culture models. Testing new drug candidates in these specific disease models is expected to decrease the lack of efficacy in patients. In addition, very rare diseases or genetic diseases for which an animal model is not available can be studied. This is especially of interest for target identification in patient-specific cells or tissue, to identify a particular target group for the clinical trial. Finally, the use of an OoC with patient-specific cells can be used to decide which treatment option has the highest chance of success in, for example, cancer treatment [43]. This potentially decreases time and costs of drug development and increases the likelihood of identifying effective treatment options. Furthermore, OoC models have the potential to provide new insights in disease development and treatment profiles, due to the possibility of continuous monitoring. This can result in the discovery and validation of new biomarkers relevant for subsequent clinical studies. During the clinical phase, OoC models can also be further applied to test hypotheses when *in vivo* results deviate from *in vitro* findings.

Despite all advantages and opportunities, OoC models for ADME/PK have, as of yet, not been applied routinely in drug development. This is mainly due to issues with availability, characterization, reproducibility, standardization, throughput, and unknown predictive performance [44–46]. There are initiatives to make OoC models common practice.

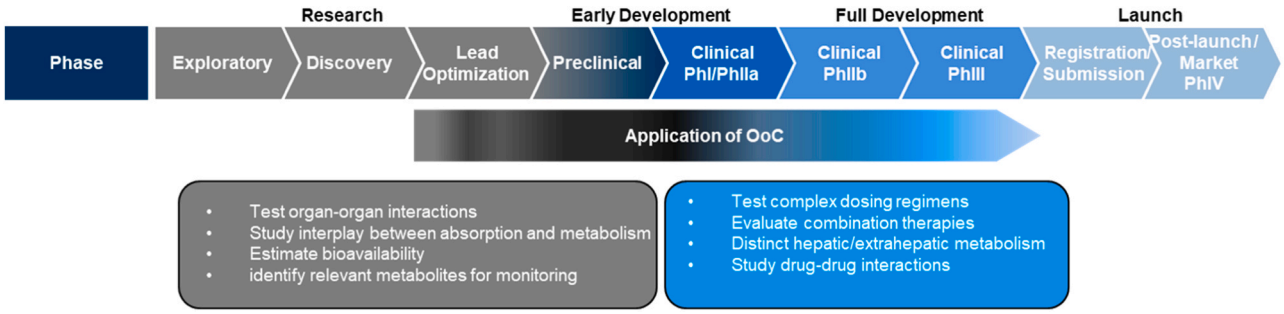

Fig. 1. Application of OoC models for PK/ADME in the drug development pipeline.

The North American 3Rs collaborative for example provides a database with all commercially available microphysiological systems, as well as enabling technologies [47].

2.3. OoC models applied in PK studies

The ultimate goal of ADME studies is to determine the behavior of a compound in the human body, preferable under healthy and disease conditions. Single OoC models are a valuable tool to study tissue-specific metabolism, clearance and toxicity. These models can be used to obtain organ PK parameters (*e.g.* intrinsic clearance) that might be scaled by modelling to estimate *in vivo* PK parameters. However, multi-OoC enable an enhanced assessment of organ-organ interactions, complex DDI and human PK predictions.

Several multi-OoC models have demonstrated the application of multi-OoC models for PK studies, which is summarized in Table 1. All studies use cell lines and primary cells, which are known to lose characteristics like transporter and metabolic enzymes function in culture over time [30]. Interestingly, multiple studies did show higher enzymatic drug metabolism when liver-OoC was combined with intestine-OoC compared to the single OoC [48–54]. This could be a result of the contribution of the intestine to first-pass metabolism, but might also indicate a positive feedback due to organ-organ interaction. The latter is highly relevant for studying DDI, which has so far not been demonstrated in any of the published systems.

For demonstrating the applicability of the models, a variety of compounds have been used impairing direct comparison of the outcomes. Most models show PK parameters for one or two compounds, but it would be most interesting to also see the performance of the model for different classes of drugs and after exposure to several drugs simultaneously. The use of in-house developed frameworks with variation in design further compromises direct comparison of outcomes, with only two studies using commercially available platforms [53,55].

Besides measurements of transport of the test compound, most studies reported in Table 1 also include metabolite formation as read-out. From a safety point of view, it is important to not only estimate systemic concentrations of parent drug, but also of the (potential toxic) metabolites. And for pro-drugs, which are activated by intestinal and liver metabolism, it is even crucial to determine whether their metabolites are able to reach target sites in sufficient levels before being eliminated from the body. Other general read-outs used are barrier integrity (TEER), metabolic capacity (urea and albumin production), cellular viability (calcein-AM) and CYP450 enzyme activity.

The majority of the multi-OoC models listed in Table 1 focus on the interaction of the intestine with the liver, to evaluate absorption and first pass metabolism. Importantly, the potential excretion of drugs and/or metabolites into bile was not included in the platforms reported. Therefore, the predicted intrinsic clearance (Cl_{int}) appears to be less accurate for compounds for which *in vivo* clearance is likely rate-determined by active hepatic uptake prior to biliary excretion [14]. To improve *in vitro* to *in vivo* translatability, a multi-OoC model for ADME studies should enable elimination of the compound *via* bile flow as well. In addition, excretion by the kidney into urine is lacking. These parameters are important determinants of drug disposition after oral intake and are required to obtain PK data translatable to human *in vivo*. Finally, biliary excretion causes compounds to end-up in the intestinal lumen where they can be re-absorbed and enter the systemic circulation again. This entero-hepatic circulation influences the half-life of drugs and may result in multiple plasma peaks. It would be a breakthrough if these processes could be accurately predicted *in vitro* with multi-OoC models.

3. Minimal viable platform for a multi-OoC model to study PK

With the fast-emerging developments in the OoC field, the main challenge to date is how to evaluate whether an OoC model is applicable for PK studies. The wide range of different technologies makes direct comparison between the systems challenging. And most models published so far demonstrate a proof-of-principle rather than a complete validation. Regulatory bodies have not yet determined which test criteria OoC models should display for acceptance, but several initiatives aim to accelerate the implementation of human focused new approach methodologies (NAMs) in the drug developmental pipeline [60,61]. Validation should at least include biological relevance, reproducibility and relevant quality controls. Traditional validation methods might not be applicable for this new technology and a fit-for-purpose validation might be more relevant. Here, the main focus is on the functionality of the model: is the *in vitro* organ model able to replicate the structural and functional mechanisms of a specific organ? And is the multi-OoC able to accurately predict PK profiles of reference compounds with known *in vivo* profiles? The highest translational value of a model is obtained by a combination of accurate physiological characteristics and clinically relevant read-outs.

3.1. Physiological requirements

PK studies need to include the most relevant organs that determine ADME of a compound, *i.e.* intestine, liver and kidney, connected in a biologically relevant environment. Further increasing the number of tissues in the model will also increase the technical complexity of the model, which becomes impractical for industrial implementation. The physiological characteristics required for PK studies are depicted in Fig. 2.

The human intestine forms a selective barrier that determines what enters the human body. Orally administered drugs need to pass the intestinal wall, a membrane composed of a mucus layer, various epithelial cell types and stromal cells. Compounds can pass the membrane *via* passive diffusion (paracellular or transcellular) or *via* active, transporter-mediated processes. Intestinal epithelial cells contain uptake transporters such as peptide transporter 1 (PEPT1), organic cation transporters (OCTs) and, organic anion-transporting polypeptide (OATPs), and the efflux transporters p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) [62] (Fig. 2A). Some transporters are expressed along the whole intestinal tract, while others are located in a

Table 1
Overview of multi-OoC models with demonstrated application in PK studies.

Organ	Cell source	Chip design	Compound	Read-out	Main results	Reference
Intestine Liver	Caco-2 HepG2 Primary human hepatocytes	In-house developed framework (PDMS) with polycarbonate membranes: Integrated Insert in Dynamic Microfluidic Platform (IIDMP)	Acetaminophen	Barrier integrity (TEER) Barrier permeability (lucifer yellow & R123) Cellular viability (resorufin) Transport Decrease compound & metabolite formation PBPK modelling	Platform for the evaluation of intestinal first-pass metabolism and liver metabolism. In intestine-liver combination higher metabolite formation observed.	Prot et al., 2014 [48]
Intestine Liver	Caco-2 TC7 Primary human hepatocytes	In-house developed framework (PDMS) with polycarbonate membranes: Integrated Insert in Dynamic Microfluidic Platform (IIDMP)	Phenacetin Omeprazole	Barrier integrity (TEER) Metabolic capacity (albumin production) Adsorption Decrease compound & metabolite formation PBPK modelling	The intestine-liver combination resulted in a decreased intestinal barrier integrity and higher metabolic activity of the hepatic CYP1A2 when compared to intestine- and liver-only.	Bricks et al., 2015[49]
Intestine Liver Skin Kidney	Primary human intestinal epithelial cells HepaRG Primary human hepatic stellate cells Human juvenile prepuce RPTEC/TERT-1	In-house developed framework (PDMS)	-	Tissue viability (LDH release) Metabolic activity (glucose & lactate) Staining qPCR Barrier integrity (TEER)	Establishment of an *in vitro* system for microfluidic ADME profiling, repeated dose and systemic toxicity testing of drug candidates over 28 days	Maschmeyer et al., 2015[56]
Intestine Liver	Caco-2 Primary human hepatocytes and non-parenchymal cells	In-house developed chip (3D printed polymer) with polycarbonate membranes	-	Barrier integrity (TEER) Cell viability (AST) CYP450 enzyme activity (CYP1A1 & CYP3A4) Metabolic capacity (urea & albumin production)	Both tissues matured independent of each other before being combined for 14 days of co-culture. Metabolic rates of hepatocytes in combined chip similar to liver-only CYP activities significantly higher compared to liver-only	Esch et al., 2016 [50]
Intestine Liver	Caco-2 HepG2	In-house developed framework (PDMS)	Apigenin	Staining (calcein-AM, f-actin) Flurorescein permeability CYP450 enzyme activity (CYP3A4) Decrease compound & metabolite formation	Apigenin metabolite profile in intestine-only different from metabolites in intestine-liver combined	Choe et al., 2017 [57]
Intestine Liver	Caco-2-BBe HT29-MTX Primary monocyte-derived dendritic cells Primary human hepatocytes and Kupffer cells	In-house developed framework (polysulfone plastic and clear acrylic) with polycarbonate membranes	Diclofenac Hydrocortisone	Barrier integrity (TEER) Mucin production Metabolic capacity (albumin production) Apparent permeability (P_{app}) Compound & metabolite profile	Model-based analysis of the experimental PK data, resulting in an estimation of diclofenac and hydrocortisone permeability and clearance. Clearance differed significantly between intestine- or liver only compared to intestine-liver combination.	Tsamandouras et al., 2017[51]
Intestine Liver	Primary human intestinal epithelial cells Primary human intestinal myofibroblast HepG2 C3A	In-house developed framework (polycarbonate, silicone and stainless steel) with polycarbonate membranes	Panadol Mannitol Caffeine	Staining Cell viability assay (calcein-AM) Apparent permeability (P_{app}) Metabolic capacity (urea & albumin production) CYP activity	P_{app} values of drug compounds in intestine-on-chip comparable to Caco2 in transwells CYP activities were significantly increased in the co-culture intestine–liver system compared to the single-organ fluidic culture system.	Chen et al., 2018 [52]
Intestine Liver Endometrium Lung Heart Pancreas Brain	Caco-2-BBe1 HT29-MTX-E21 Primary human hepatocytes Kupffer cells Human endometrial epithelial adenocarcinoma Primary human bronchial epithelial cells Human iCell	In-house developed framework (polysulfone plastic and clear acrylic) with polycarbonate membranes: Physiome-on-a-Chip	Diclofenac	Barrier integrity (TEER) Metabolic capacity (albumin production) Compound & metabolite profiles	A platform showing reliable, robust operation and maintenance of phenotypic functions for 3 weeks with continuous interaction, as well as PK analysis of diclofenac metabolism	Edington et al., 2018[58]

(*continued on next page*)

Table 1 (*continued*)

Organ	Cell source	Chip design	Compound	Read-out	Main results	Reference
	cardiomyocytes 2 Rat pancreatic islets Human neural progenitor cells					
Intestine Liver	Caco-2 HT-29 HepaRG HHSteC	Two-Organ-Chip platform (TissUse GmbH).	Acetaminophen	Cell viability (MTT, staining) qPCR (albumin, enzymes) Decrease parent compound	Intestine-only and intestine-liver combined showed slower acetaminophen absorption than reported *in vivo*, irrespective of flow. Intestine-liver combined system demonstrated metabolic activity, in contrast to liver-only.	Marin et al., 2019[53]
Intestine Liver	Caco-2 cells HepaRG cells	In-house developed framework (PDMS) with polycarbonate membranes	Triazolam	Decrease compound & metabolite formation PBPK modelling	Clearance of glucuronidation metabolites in the intestine-liver combined system was higher than that in the single culture system. The plasma concentration profile of triazolam and its hydroxy metabolites in humans could be quantitatively simulated.	Arakawa et al., 2020[54]
Intestine Vasculature Liver Kidney	Caco-2-BBe HUVECs Primary human liver sinusoidal microvascular endothelial cells Primary human hepatocytes Primary human glomerular microvascular endothelial cells	In-house developed framework (PDMS), with polycarbonate membranes. Linkage *via* automatic liquid transfer	Nicotine	Barrier function (Inulin-FITC) Apparent permeability (P_{app}) Metabolic capacity (albumin production) CYP450 enzyme activity (CYP3A4) PBPK modelling	The platform enabled predictions of drug ADME and clinical PK/PD parameters. Addition of an arterial volume reservoir volume to account for missing organs and tissues (*e.g.* fat) and for drug specific volume of distribution	Herland et al., 2020[41]
Intestine Vasculature Liver Kidney	Caco-2 HUVEC HepG2 HK-2	In-house developed framework (PDMS), with polycarbonate membranes.	Ginsenosides compound K	Cell viability (Calcein-AM, CCK-8) Cell differentiation (ALPi activity) Barrier function (propranolol, sodium fluorescein) Metabolic activity (cyclophosphamide) Cytotoxicity (MTT) Decrease compound & metabolite formation	The pharmacokinetic results of ginsenosides compound provided by chip were consistent with previous reports, demonstrating the reliability of the organon-a-chip platform and its potential for use in pharmacokinetic studies of carbohydrate-drugs.	Liu et al., 2020 [59]
Intestine Liver	Caco-2 cells HT-29 cells Primary human hepatocytes	PhysioMimix gut–liver MPS (CN-BIO)	Mycophenolate mofetil	TEER Cell protein Medium volume Fraction unbound ($F_{u,inc}$) Decrease compound & metabolite formation PBPK modelling	Determination of the relative contribution of intestine only, liver only and intestine-liver combination to metabolism of mycophenolate mofetil. Mechanistic modelling of experimental data enabled estimation of permeability and clearance for the prodrug, active drug and glucuronide metabolite.	Milani et al., 2022[55]

specific region [63]. Generally, drug permeability is considered to be higher in the duodenum and jejunum compared to the ileum and colon, but this can be influenced by intestinal transit time. Besides drug transporters, the intestinal cells also contain drug metabolizing enzymes that contribute to the first-pass metabolism of compounds, including cytochrome P450 (CYP) enzymes, uridine 5′-diphospho-glucuronosyl-transferase (UGT) and sulfotransferase (SULT) [64].

The human liver receives all compounds absorbed in the intestine *via* the portal vein before they can enter the systemic circulation. Mature hepatocytes, the most abundant cell type in the liver, have a typical epithelial cell structure, secrete albumin and produce urea and bile acids [65]. The cells express genes for transport proteins, including the uptake transporters Na^+-taurocholate co-transporting polypeptides (NTCP), organic anion transporters (OATs) and OATPs, but also the efflux transporter multidrug resistance-associated protein (MRP3). For biliary excretion, the cells express transporters like BCRP, bile salt export pump (BSEP), P-gp, multidrug and toxic compound extrusion 1 (MATE1), multidrug resistance protein 3 (MDR3) and MRP2. Being a highly metabolic active organ, hepatocytes also express a large panel of phase I metabolizing enzymes (CYPs) and phase II metabolizing enzymes, including UGTs, glutathione S-transferase (GST) and SULTs (Fig. 2B).

The human kidney is composed of subunits called nephrons, which include the glomerulus, proximal tubule, loop of Henle, distal tubule, and the collecting duct. The proximal tubule is the most relevant segment of the nephron for drug metabolism studies since this part contains the various metabolic enzymes and transporters that affect the excretory functions of the kidney. The transporter proteins include OATs, OCTs, urate transporter 1 (URAT1), MATE proteins and megalin/cubilin that facilitate endocytosis (Fig. 2C). UGT is a key metabolic enzyme in the kidney, but CYP enzymes and GST are also expressed in the proximal tubule [45,66].

Key features of an accurate multi-OoC for PK studies are (1) physiological relevant transporter and enzyme function for all relevant organs, (2) polarization of the cells and (3) separated compartments with

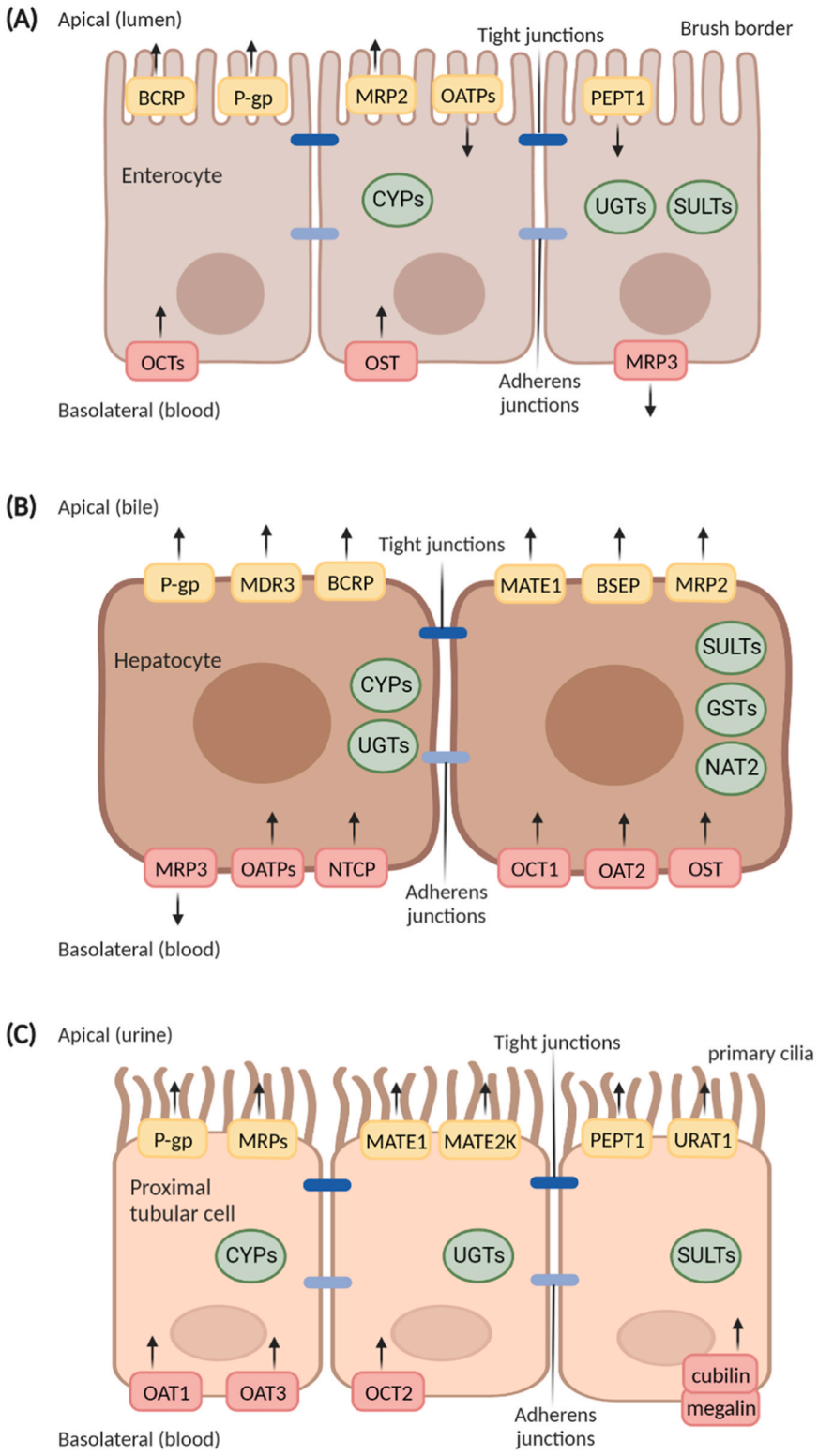

Fig. 2. Physiological parameters required for PK studies. Polarization, key drug transporters and drug metabolizing enzymes for (A) intestine, (B) liver and (C) proximal tubule. Apical transporters indicated in yellow, basolateral transporters indicated in red and metabolic enzymes indicated in green. Created with BioRender.com.

sampling options. These compartments include a luminal (intestinal) compartment, a continuous blood-flow connecting the organs, a bile compartment (with the option to re-connect to the luminal compartment to mimic entero-hepatic circulation) and a urine compartment. Ideally, the platform is modular, enabling both isolation of specific tissues/compartments and connection in a study-specific set-up. Table 2 highlights for each tissue which biological functionalities are required in the multi-OoC model: the formation of a barrier with accurate permeability, presence of differentiated cells, transport activity and metabolic capacity is key and should be comparable to human *in vivo* levels. The

Table 2
Biological characteristics required for multi-OoC models for PK studies, with associated parameters/markers to evaluate the functionality.

Tissue	Key functionality	Characteristics
Intestine	Barrier	Accurate permeability: TEER, inulin-FITC, A/A ratio[68,69] Adequate apical-basolateral polarization: presence of F-actin and integrins, presence of brush border/microvilli on the apical side Tight and adherens junction formation: E-cadherin, ZO-1 Polarization/trafficking of transporters[70]
	Differentiation	Various intestinal cell types present, with markers ALPi (enterocytes) LYZ (Paneth cells), MUC2 (goblet cells), CHGA (enteroendocrine cells), Lgr5 (stem cells)[71]
	Transport	Accurate expression and function of transporters: BCRP, P-gp, MRP2, OATP1A1/2B1, PEPT1 (apical), OCTs, OSTα/β, MRP3 (basolateral)[62,72]
	Metabolism	Accurate expression and activity of drug metabolizing enzymes: CYP1A1, CYP3A4, CYP2C9 (phase I), UGTs, SULTs (phase II)[64]
Liver	Barrier	Accurate permeability: inulin-FITC[73] Adequate apical-basolateral polarization: presence of F-actin and integrins Tight and adherens junction formation: E-cadherin, ZO-1 Polarization/trafficking of transporters[70]
	Differentiation	Accurate expression of markers for mature hepatocytes: ALB, HNF4α, CK8, CK18 (general markers), GLUL (pericentral marker), ASS1 (periportal marker) Albumin production[74]
	Transport	Accurate expression and function of transporters: P-gp, MDR3, BCRP, MRP2, MATE1, BSEP (apical), MRP3, NTCP, OATP1B1, OATP1B3, OATP2B1, OCT1, OAT2, OSTα/β (basolateral) Bile excretion[72]
	Metabolism	Accurate expression and activity of drug metabolizing enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2D6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4 (phase I), UGTs, SULTs, GSTs, NAT2 (phase II)[75,76]
Kidney (proximal tubule)	Barrier	Accurate barrier formation: inulin-FITC[77] Adequate apical-basal polarization of cells: presence of F-actin and integrins, presence of primary cilia Tight and adherens junction formation: E-cadherin, ZO-1 Polarization/trafficking of transporters: Na^+K^+ATPase pump[78]
	Differentiation	Recapitulation of proximal tubule physiological functions: reclamation of glutathione, reabsorption of glucose and albumin, ammoniagenesis and vitamin D metabolism[78,79]
	Transport	Expression and functionality of transporters: MATE1/2 K, MRP2, MRP4, P-gp, PEPT1/2, URAT1 (apical), OAT1, OAT3, OCT2 (basolateral)[72] Functional megalin/cubilin mediated uptake *via* endocytosis[80]
	Metabolism	Expression and functionality of metabolizing enzymes: CYP2B6, CYP3A5, CYP3A4 (phase I), UGT1A6, UGT1A9, UGT2B7, SULTs (phase II)[75,76]

ALB = albumin, ALPi = intestinal alkaline phosphatase, ASS1 = argininosuccinate synthetase 1, A/A ratio = antipyrine / atenolol ratio, BCRP = breast cancer resistance protein, BSEP = bile salt export pump, CHGA = chromogranin A, CK = cytokeratin, CYP = cytochrome P450, GLUL = glutamine synthetase, GST = glutathione S-transferase, HNF4α = hepatocyte nuclear factor 4 alfa, LGR5 = leucine-rich repeat-containing G-protein coupled receptor 5, LYZ = lysozyme, MATE = multidrug and toxic compound extrusion, MDR = multidrug resistance protein, MRP = multidrug resistance-associated protein, MUC2 = mucin 2, NAT2 = n-acetyltransferase 2, NTCP = sodium taurocholate cotransporting polypeptide, OAT = organic anion transporter, OATP = organic anion-transporting polypeptide, OCT = organic cation transporter, OST = organic solute transporter PEPT = peptide transporter, P-gp = p-glycoprotein, SULT = sulfotransferase, SLC = solute carrier transporter, TEER = trans-epithelial electrical resistance, UGT = UDP-glucuronosyltransferase, ZO1 = zonula occludens-1

transporters with highest clinical relevance for evaluation during drug development in the intestine, liver and kidney are extensively discussed elsewhere [67].

3.2. ADME specific read-outs

The complexity of a multi-OoC model offers a wide range of potential measurements and read-outs. The final selection of parameters evaluated needs to be biologically and clinically relevant, reliable, sensitive and translatable.

Electrical measurement of the impedance is the most-used method for assessing the barrier integrity of a cell monolayer *in vitro*, as it represents cell layer confluency and tight junction formation. Trans-Epithelial Electrical Resistance (TEER) values for both tissue and cell layers are extensively reported in the literature, allowing comparison with new systems. Furthermore, it can be monitored continuously during culture and experiments. However, it should be considered that TEER measurements are prone to fluctuation due to temperature, medium composition, accuracy or geometry of the electrodes used [81]. In addition, not all OoC systems allow incorporation of electrodes on both sides of the cell monolayer. Other methods to evaluate barrier integrity make use of large inert fluorescently labelled compounds (*e.g.* inulin-FITC or dextran-FITC) with low permeability, that exclusively pass a barrier *via* paracellular diffusion. The combination of a moderately permeable drug with a high permeable drug provides even more accurate assessment of barrier integrity. For example, atenolol (F_a ≈50%) passes a barrier *via* the paracellular route, while antipyrine (F_a =100%) passes transcellularly [82]. Transport is expressed as apparent permeability (P_{app}) value (Eq. (1)), which depicts the degree of transport of a compound (dQ) corrected for time (dT), surface (A) and concentration (C0). A transcellular over paracellular transport ratio ≥ 2 (Eq. (2)) indicates accurate barrier integrity [69,83].

$$^{\circ}P_{app} = \frac{\frac{dQ}{dT}}{A \times C_0} \quad (1)$$

$$A/A \text{ ratio}^{\circ} = \frac{P_{app}{}^{\circ}\text{Antypirine}}{P_{app}{}^{\circ}\text{Atenolol}} \quad (2)$$

For each cell type included, the level of differentiation can be determined by the parameters provided in Table 2. The cellular composition needs to reflect *in vivo* tissue composition. In addition, for all OoC modules it is necessary to evaluate cellular viability after long-term culture, by for example methyl thiazolyl tetrazorium assay (MTT), lactate dehydrogenase (LDH) release or ATPase activity. Clinically applied biomarkers to screen for potential DDI that can also be monitored in multi-OoC models include bilirubin, coproporphyrin and creatinine levels [84].

To assess accurate levels of transport and metabolism, most relevant markers for each organ need to be determined as also provided in Table 2. Expression levels need to be evaluated on both gene and protein level. Subsequently, accurate activity of the transporters and enzymes should be demonstrated by measuring P_{app} values of relevant compounds. For all transporters and enzymes, specific substrates and inhibitors/inducers are described in literature [62,85,86]. Besides P_{app} values, the efflux ratio of a compound is informative to evaluate transporter activity and localization. An efflux ratio ≥ 1 indicates active efflux to one specific side of the barrier (Eq. (3)).

$$\text{Efflux Ratio} = \frac{P_{app}\ \text{basolateral} > \text{apical}}{P_{app}\ \text{apical} > \text{basolateral}} \quad (3)$$

Oral bioavailability (F) of a drug candidate, the fraction that reaches the systemic circulation intact, can be calculated with the fraction absorbed (F_a), fraction escaping metabolism in the intestine (F_g) and the fraction escaping metabolism in the liver (F_h) (Eq. (4)). For the prediction of first-pass effects in the intestine and liver the intrinsic clearance (CL_{int}) is typically measured in *in vitro* systems of the respective organ. During early drug development stages, the CL_{int} is determined from the metabolic depletion of parent compound over time. Hepatic clearance for example (CL_h) can be calculated based on the extraction rate (E) corrected for hepatic blood flow (Q_h) (Eq. (5)). The sum of the individual organ clearance values is equal to the systemic clearance.

$$F = F_a \times F_g \times F_h \quad (4)$$

$$Cl_h = Q_h \times E \quad (5)$$

Table 3
Reference compounds for the validation of multi-OoC models to study ADME/PK profiles.

Drug	Human bioavailability	Intestine	Liver	Kidney	Relevance
Coumarin[97,98]	Low oral bioavailability (4%)	Transport by BCRP Metabolism by UGTs and SULTs	Transport of the metabolites by MRP3 and MRP4 (basolateral). Metabolism by CYP2A6 and UGTs.	Transport of the metabolites by MRP3 and MRP4 (basolateral). 80–90% of metabolites excreted *via* urine	Extensive first-pass metabolism (intestine and liver) Predominant excretion of metabolites *via* the kidneys
Acyclovir[99,100]	Low oral bioavailability (10–20%)	-	-	Transport by OAT1, OAT2, and OAT3 (basolateral), and MATE1 (apical) Metabolism by ALDH1A, ALDH2 and AOX1 F_e = 62–91%	Minor metabolism in kidney Mainly cleared by active renal secretion
Rosuvastatin[90,91]	Low oral bioavailability (20%)	Transport by BCRP, OATP2B1	Transport by OATP1B1, OATP1B3 and OATP2B1 (basolateral), and BCRP, MRP2 and BSEP (apical) Metabolism by CYP2C9 and CYP2C19 (10%) Excretion *via* feces (90%)	Transport by OAT1 and OAT3 (basolateral) Excretion *via* urine (10%)	Minimal metabolism, no DDIs. Mainly cleared *via* biliary excretion
Phenacetin[101,102]	Low to moderate oral bioavailability (19–49%)	Metabolism by UGTs and SULTs	Transport of the metabolites by MRP3 and MRP4 (basolateral), and BCRP and MRP2 (apical) Metabolism by CYP1A2, CYP2E1, UGTs, SULTs and GSTs	Metabolism by UGTs and SULTs	Metabolism in intestine, liver and kidneys
Diclofenac[91,103]	Moderate oral bioavailability (50–90%)	Metabolism by CYP2C9 and UGT2B7 F_a = 1.0 F_g = 0.64	Transport by OAT2 (basolateral). Transport of the metabolites by MRP3 (basolateral), and MRP2 and BCRP (apical) Metabolism by CYP2C9 and UGT2B7 F_h = 0.85	Transport of the metabolites by OAT1, OAT2 and OAT3 (basolateral), and MRP2, BCRP and OAT4 (apical)	Mainly cleared by first-pass metabolism
Metformin[89,104]	Moderate oral bioavailability (55%)	Transport by OCTs (basolateral) F_a = 0.55	Transport by OCTs (basolateral)	Transport by OCT2 (basolateral), MATE1/2 K (apical)	Mainly cleared by active renal secretion
Midazolam[91,105]	Moderate oral bioavailability (66%)	Metabolism by CYP3A4 F_a = 1.0 F_g = 0.37	Metabolism by CYP3A4 and UGTs F_h = 0.33–0.76	F_e < 0.5% Excretion of metabolites *via* urine (70%)	Mainly cleared by first-pass metabolism
Digoxin[92]	High oral bioavailability (70–80%)	Transport by P-gp (apical)	Transport by OATP1B3 (basolateral) and P-gp (apical)	Transport by OATP4C1 (basolateral) and P-gp (apical) F_e = 50–70%	High affinity substrate for efflux transporter P-gp Predominantly excreted *via* the kidneys
Mycophenolate mofetil[94,106]	High oral bioavailability (81–94%)	Metabolism by CES2 and UGTs	Transport of the metabolites by OATP1B1 and OATP1B3 (basolateral), and MRP2 (apical) Metabolism by CES1, CES2, CYP3A4 and UGTs	Transport of the metabolites by OAT1 and OAT3	Described entero-hepatic circulation
Cyclosporin A[96,107] or Semaglutide [108]	Variable[109,110]	Metabolism by peptidases and beta-oxidation	Metabolism by CYPs	Metabolism by peptidases and beta-oxidation	Mainly extrahepatic catabolism. Metabolites predominantly excreted *via* the kidneys
Mipomersen[111]	Not applicable	unknown	Transport by ASPGR, metabolism by nucleases	unknown	Transport *via* endocytocis Degradation by endonucleases and exonucleases

ALDH = aldehyde dehydrogenase, ASPGR = asialoglycoprotein receptor, AOX = aldehyde oxidase, BCRP = breast cancer resistance protein, BSEP = bile salt export pump, CES = carboxylesterase, CYP = cytochrome P450, DDI = drug-drug interactions, F_a = fraction absorbed, F_e = fraction excreted unchanged in urine by the kidney, F_g = fraction escaping metabolism in the intestine, F_h = fraction escaping metabolism in the liver, GST = glutathione S-transferase, MATE = multidrug and toxic compound extrusion, MRP = multidrug resistance-associated protein, OAT = organic anion transporter, OATP = organic anion-transporting polypeptide, OCT = organic cation transporter, P-gp = p-glycoprotein, SULT = sulfotransferase, UGT = UDP-glucuronosyltransferase.

With increasing knowledge about the metabolites formed and the availability of metabolite standards or radiolabeled test compounds, intrinsic clearance can also be determined by the detection of specific metabolites in the medium, which is especially useful for compounds with low metabolic turnover. If the enzymes involved in the metabolism are known, the fractional contribution of the different metabolic pathways to the overall intrinsic clearance could be estimated by the formation of the metabolites [87]. Ideally, measurements in the model allow to distinguish between clearance by metabolism or clearance by direct excretion into bile. In general, the liver is perceived as the major metabolism organ for most small molecular drugs. The kidney has an important role in drug and metabolite elimination, not only for small molecule drugs but also for peptides and proteins. Simulation of intravenous dosing instead of oral administration will be required for these type of compounds, which should be modeled with a modular multi-OoC model.

3.3. Reference compounds to validate multi-OoC models for PK studies

The selection of compounds to demonstrate functionality is critical for validation of multi-OoC models. For toxicology studies, the EURL ECVAM library of reference chemicals provides a database that can be used to standardize, qualify, characterize and compare *in vitro* and *in silico* models [88]. However, to the best of our knowledge, such database currently does not exist for ADME/PK studies. Table 3 provides a list with suggestions for relevant reference compounds with described human ADME profiles to enable correlations and evaluation of the predictive value of a multi-OoC model. Compounds are listed from low to high human oral bioavailability, with the specific ADME/PK processes that can be validated with it. To allow comparison with *in vivo* data, it is important to test pharmacologically relevant drug concentrations. However, for *in vitro* dosing certain concentrations cannot be exceeded otherwise there is saturation of the system and specificity will be lost [85]. This should be taken into account and can be predicted prior to the experiment with computational modeling.

For transport validation, it is important to select compounds with diverse characteristics, *e.g.* low and high permeability, since some OoC models might only be applicable to a specific class of compounds. When testing a novel chemical entity, the model should be at least validated with a reference compound with similar characteristics. As internal standard, this reference can be included in the new tests as well, but physical, chemical or permeation interactions should be avoided.

Compounds with low metabolic clearance are challenging to assess with standard tools, and therefore highly relevant to assess in multi-OoC models for ADME/PK. Metformin is an interesting reference compound to measure transport, since it is absorbed in the intestine and distributed throughout the body without being metabolized. The compound is excreted unchanged in the urine, making active tubular secretion the principle route of metformin clearance from the body [89]. Rosuvastatin is another compound with known low bioavailability (20%) and minor metabolism by the liver (10%). The majority of this compound (90%) is excreted *via* feces, while 10% is eliminated from the body *via* the urine [90,91]. Validation of specific transporters can be performed by well-characterized drug compounds, for example digoxin that is a high affinity substrate for the efflux transporter P-gp [92].

The metabolic activity of tissues in OoC models can be examined by commercially available kits that measure the relative formation of luminescent products in cells exposed to pro-luminescent substrates specific for the enzymes. However, it is preferred to expose the cells to compounds specific to the enzymes, followed by measuring metabolite formation using LC-MS [85]. Table 3 includes reference compounds with described specificity for metabolic enzymes. Midazolam, for example, is extensively used both *in vitro* and *in vivo* for the prediction of CYP3A-mediated DDIs [93]. The compound is mainly cleared from the body by first-pass metabolism in the intestine and liver. Coumarin is another compound with extensive phase I and phase II metabolism occurring in the intestine and liver, and subsequent active clearance of metabolites by the kidneys (Table 3). When evaluating activity of metabolic enzymes, selective inhibitors like probenecid, ketoconazole, rifampicin and cimetidine should also be included. Finally, it is of added value to also demonstrate the potential to induce CYP enzyme and transporter activity in the system, making the model also applicable for studying CYP-specific DDIs. The FDA provides an extensive list of clinically relevant substrates, inhibitors and inducers [93].

Mycophenolate mofetil is an interesting reference compound to validate the enterohepatic circulation in multi-OoC models. The route of metabolism for this prodrug is well characterized, with bioactivation in the intestine and liver by CES1 and CES2 upon oral administration, and glucuronidation by UGTs. One metabolite is mainly excreted in the urine by active tubular secretion, while another metabolite is excreted by the liver in the bile *via* MRP2 [94]. PK studies demonstrated a secondary peak of this metabolite in plasma 6–12 h after oral administration, indicating enterohepatic circulation consisting of microbial breakdown of the glucuronide releasing the unconjugated mycophenolate that, subsequently, can be reabsorbed from the intestinal lumen [95].

Validation of biotherapeutics in multi-OoC models is more challenging since there is limited literature available on ADME properties for these entities. Peptides are rarely administered orally due to instability and degradation in the gastro-intestinal tract. They are mostly cleared from the body *via* the kidneys or proteolytic hydrolysis, often considered extrahepatically but the identity of the involved drug metabolizing enzymes is still unknown for most peptide drugs [96]. For these drugs, cyclosporin A or semaglutide can serve as reference compounds (Table 3). Antisense oligonucleotides have completely unique ADME characteristics that are not fully defined [18]. They are never administered orally, but with a modular multi-OoC system other routes of administration like intravenous injection could also be mimicked. Mipomersen is a relevant antisense oligonucleotide to test in multi-OoC models for ADME/PK (Table 3), to validate endocytosis and nuclease function in the system.

3.4. Translation to in vivo profiles

Although multi-OoC models can capture key biological characteristics, they never completely represent the complexity of a human body. In addition, the size and volume of the tissues and compartments in the models require scaling to human dimensions. As a result, findings of *in vitro* ADME/PK models cannot directly be compared to human data. One study that comes close used a multi-OoC model to evaluate PK and toxicity of terfenadine, trimethylamine, and vitamin [112]. In this study, the authors used a medium-transfer method to couple different OoC models representing the intestine, liver, kidney and the blood-brain barrier and found similarities between the experimental and clinical data outcomes. Furthermore, the authors describe that scaling is one of the major challenges when coupling organs *in vitro*. Therefore, mechanistic modeling of the experimental data will likely be a valuable asset. *In silico* physiologically based PK (PBPK) modelling provides options to apply physiological relevant scaling, to include factors that are missing in the *in vitro* model(s) and to correct for specific *in vivo* characteristics, all leading to improved *in vitro* to *in vivo* extrapolations (IVIVE) [113]. For example, a correction factor is needed for the ratio of intestinal to liver to kidney cells in a multi-OoC model. The intestine has a larger surface area compared to the liver *in vivo*, but absorption of the drug might only occur in a dedicated region. Also, blood flow rates are different across organs. Simple scaling based on organ weight fails to capture how organ functions scale with respect to each other [32], but mathematical modelling can account for *in vitro / in vivo* mismatched organ size. Recent publications demonstrate that OoC models show an enhanced predictability when combined with *in silico* data, compared to experimental data only [9,14]. Implementing *in silico* analysis early in drug development may also guide experimental designs, provide model-informed selection of drug candidate concentrations to study *in*

vitro and translate experimental data to clinical outcomes [13]. Important issues to consider when implementing PBPK modelling are model construction, parameter estimation and validation strategies [9].

Experimental data required for IVIVE:

- **Recovery**. Non-specific binding and stability of the compound in the model.
- **Number of active cells**. For quantitative translation.
- **Apparent permeability (P_{app}) value**. The P_{app} value represents an interplay between absorption, excretion and metabolism. Transport is corrected for area of exposure.
- **Intrinsic clearance (CL_{int})**: Metabolic turnover of parent compound over time.
- **Metabolite profiles**. Levels of primary and secondary metabolites in different tissue compartments provide information on the route of elimination.

4. Conclusion

Organ-on-chip PK models are advanced *in vitro* models that have the potential to improve the prediction of human oral bioavailability and intrinsic clearance of drug candidates. By replicating human biology and physiology, these models can capture specific characteristics required for ADME/PK studies, thereby bridging the gap between conventional *in vitro* and *in vivo* models. Combined with *in silico* PBPK modeling, multi-OoC models can provide PK parameters during the preclinical phase of drug development that will lead to improved clinical trials. However, despite the advantages and opportunities, OoC models have not yet been routinely applied in drug development. The field of OoC is relatively new and still in development, and key elements required for further development were discussed in this review. Once the predictive performance of multi-OoC models is validated and issues related to availability, reproducibility and standardization are resolved, they can ultimately facilitate more efficient drug discovery in future.

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CRediT authorship contribution statement

Marit Keuper-Navis: Conceptualization, Investigation, Writing – original draft. **Markus Walles**: Conceptualization, Writing – review & editing. **Birk Poller**: Conceptualization, Writing – review & editing. **Adam Myszczyszyn**: Writing – review & editing. **Thomas K. van der Made**: Writing – review & editing. **Joanne Donkers**: Writing – review & editing. **Hossein Eslami Amirabadi**: Writing – review & editing. **Martijn J. Wilmer**: Writing – review & editing. **Saskia Aan**: Writing – review & editing. **Bart Spee**: Supervision, Conceptualization, Funding acquisition, Writing – review & editing. **Rosalinde Masereeuw**: Supervision, Conceptualization, Funding acquisition, Writing – review & editing. **Evita van de Steeg**: Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

None of the authors have competing / financial or personal interests on the content of this invited review manuscript.

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