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Integration of *in silico* and *in vitro* platforms for pharmacokinetic–pharmacodynamic modeling

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Importance of the field: Pharmacokinetic–pharmacodynamic (PK-PD) modeling enables quantitative prediction of the dose–response relationship. Recent advances in microscale technology enabled researchers to create *in vitro* systems that mimic biological systems more closely. Combination of mathematical modeling and microscale technology offers the possibility of faster, cheaper and more accurate prediction of the drug's effect with a reduced need for animal or human subjects.

Areas covered in this review: This article discusses combining *in vitro* microscale systems and PK-PD models for improved prediction of drug's efficacy and toxicity. First, we describe the concept of PK-PD modeling and its applications. Different classes of PK-PD models are described. Microscale technology offers an opportunity for building physical systems that mimic PK-PD models. Recent progress in this approach during the last decade is summarized.

What the reader will gain: This article is intended to review how microscale technology combined with cell cultures, also known as 'cells-on-a-chip', can confer a novel aspect to current PK-PD modeling. Readers will gain a comprehensive knowledge of PK-PD modeling and 'cells-on-a-chip' technology, with the prospect of how they may be combined for synergistic effect.

Take home message: The combination of microscale technology and PK-PD modeling should contribute to the development of a novel *in vitro/in silico* platform for more physiologically-realistic drug screening.

Keywords: ADME, cell culture, microfluidics, pharmacokinetic–pharmacodynamic model

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1. Introduction

After administration, drugs go through complex, dynamic processes involving absorption, distribution, metabolism and elimination, collectively known as ADME [1,2]. Changes in ADME, that is, pharmacokinetics (PK), may lead to changes in drug effect and response, often resulting in unwanted toxic side effects or reduced efficacy. In fact, unforeseen toxicity and the lack of efficacy have been cited as the primary reasons behind the high attrition rate in the drug development process, accounting for ~ 60% of failures [3]. Current *in vitro,* cell-based assays are far from a complete model of the human body, as these assays only mimic steady-state conditions. In the human body, on the other hand, target cells are exposed to a time-dependent concentration profile of a drug and its metabolites as a result of the complex ADME occurring in multiple organs. While many *in vitro* methods have been developed in an attempt to predict the ADME properties of drug candidates [1,2], these *in vitro* methods are often incomplete models of PK *in vivo,* and animal or human studies are indispensible steps in the drug development process before final approval.

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Article highlights.

- Recent development in combining microfabrication and cell culture technology has brought a novel *in vitro* platform that can reproduce the pharmacokinetics (PK) of drugs.
- Various forms of PK and pharmacodynamic (PD) models are available, with different levels of complexity. An integration of pharmacokinetic–pharmacodynamic (PK-PD) model allows prediction of pharmacological effect from a given dose.
- PK-dependent effects of drugs have been observed using microscale cell culture systems.
- Authentic reproduction of organ functions is essential for developing a realistic *in vitro* model of a drug's PK-PD. The recent development in microscale technologies has resulted in novel devices that can reproduce the organ functions more faithfully than conventional devices.

This box summarizes key points contained in the article.

A mathematical modeling approach, often known as an integrated pharmacokinetic–pharmacodynamic (PK-PD) modeling, strives to predict the relationship between the drug dose and the final pharmacological effect, and its role in drug development has been well recognized [4,5]. There has been a significant amount of improvement in this modeling approach, especially in terms of the transition from an empirical approach towards mechanism-based mathematical models [6]. However, PK-PD modeling can benefit from further improvement to gain even broader acceptance by pharmaceutical industry than now. To this end, several obstacles need to be overcome, such as lack of human data and the improvement of translational research across species [7].

The recent development of microfabrication technology and its integration with cell culture techniques have allowed development of novel microscale devices that are capable of performing experiments in a manner that has not been possible with conventional, macroscale systems [8,9]. Although not realized widely yet, these advances are beginning to show a potential for complementing and enhancing the PK-PD modeling approach. Among the possibilities, the ability to fabricate and operate devices with complex, microscale structures offers the possibility of creating an experimental platform that can reproduce multi-organ interactions *in vitro.* A microfluidic device can be fabricated with multiple chambers, representing different organs, which are then connected with fluidic conduits representing the blood flow. Such a device can work as a physical realization of a mathematical, PK-PD model, which we have termed a 'microscale cell culture analog (μCCA)' [10]. Being a physical realization of a PK-PD model, the μCCA can be designed and fabricated based on the mathematical model, and then can be used to test a hypothesis of the model. A mathematical model can further be used to analyze and interpret the experimental observations, and extrapolated to predictions of human response (**Figure 1**).

In this article, we explore the potential of microscale systems in working in a combination with mathematical PK-PD models, and ultimately improve the predictability of *in vitro* toxicity assay systems. First, we briefly describe the concept of physiologically-based pharmacokinetic (PBPK) and pharmacodynamic (PD) modeling, and how these methods can link a drug dosage to a pharmacological effect. Second, we discuss the recent research efforts in the development of microscale/microfluidic systems that can test the drug's effect in a PK-PD-based way. Although there is still much to be done, we are beginning to see several interesting examples of microscale systems that examine the effect of drugs arising from multi-organ interactions. The μCCA that has been developed at Cornell is described as well as other microscale devices based on similar concepts. The essential prerequisite for such an approach to be successful is the ability to reproduce normal physiological functions of key organs. In the last section, we summarize the research effort towards 'artificial organs', with a special emphasis on the contribution of microfabrication technology to achieving this goal.

2. PK-PD modeling

We briefly discuss the concept of PK-PD modeling, its applications and limitations. This is intended to be an informative, rather than a comprehensive review of the field. Here, we cover the aspect of PK-PD modeling that is particularly pertinent to the concept of experimental platform that is discussed later. For a more in-depth review, we recommend articles that focus specifically on PK-PD modeling [4-6,11-15].

2.1 Empirical PK models

PK refers to the science of drug absorption, distribution and elimination, or more specifically the quantification of those processes, leading to the understanding, interpretation and prediction of concentration–time profiles in blood and various organs [7]. Pharmacokinetic models strive to predict the concentration–time profiles in blood or various organs from a given drug dose. Depending on the complexity of models, PK models can be divided into several categories, with two main categories being empirical models and mechanistic models.

The simplest form of an empirical PK model is a one-compartment model, which assumes that a rapid equilibrium throughout the body is achieved quickly after drug administration (**Figure 2A**). In this model, the PK can be described by a single exponential term, and the semilog plot of drug concentration versus time results in a straight line. However, many drugs do not reach equilibrium rapidly, and require time for distribution before they reach equilibrium. Such drugs can be modeled using a two-compartment model, which consists of a central compartment and a peripheral compartment (**Figure 2B**). The drug is administered to a

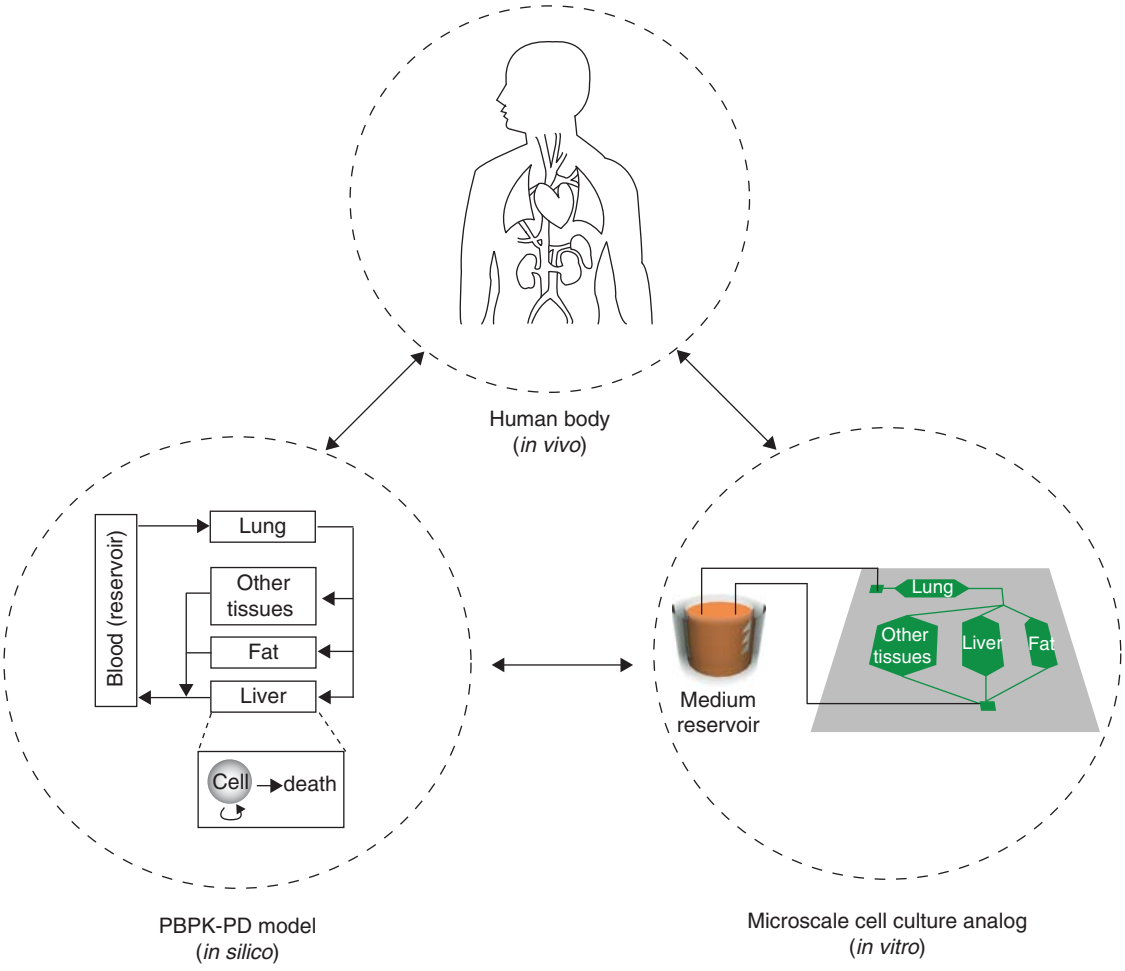

Figure 1. The concept of PK-PD modeling and a μCCA as a physical realization of a PK-PD model. A PK-PD model is constructed based on the physiology of the human body. In a μCCA, separate chambers representing organs are fabricated and connected with fluidic conduits mimicking the blood circulation.
PK-PD: Pharmacokinetic–pharmacodynamic; μCCA: Microscale cell culture analog.

central compartment, which is a lumped, hypothetical sum of blood and well-perfused organs such as the liver and kidney. A peripheral compartment is generally a sum of poorly-perfused organs, such as muscle, skin and fat. The two-compartment model is described by a sum of two exponential terms, and the semilog plot of drug concentration versus time is a biphasic straight line. Although these empirical models are mathematically simple and do not have a mechanistic basis, they are still widely used to analyze the PK of drugs due to their simplicity [16-18].

2.2 PBPK model

In a PBPK model, separate compartments are assumed for different organs, which are connected with hypothetical blood flows mimicking the blood circulation in the body (**Figure 2C**). This model is based on physiological considerations, because physiological parameters such as organ sizes and blood flow rates, and tissue–plasma partition coefficients are used to construct the model. Mass balance equations are set up to describe the flow in, flow out and reactions in the compartment (**Figure 2D**). A set of ordinary differential equations can then be solved numerically with mathematical software such as MATLAB and MATHEMATICA, as well as specialized PK modeling software packages [19].

A PBPK model has advantages over an empirical model in that it has more mechanistic basis, because it is based on the anatomic structure of the organism. Therefore, each compartment has a physiological context, which makes it possible to identify a specific mechanism of action related to certain organs. However, it should be noted that a PBPK model can be 'predominantly' mechanistic only, because there will always be an empirical aspect in the model to some extent. For example, it is quite common to lump organs with similar kinetics into one compartment; the kidney, brain and liver can be categorized as well-perfused organs, whereas muscle, skin, bone and fat are usually summed as poorly-perfused organs. Lumping of

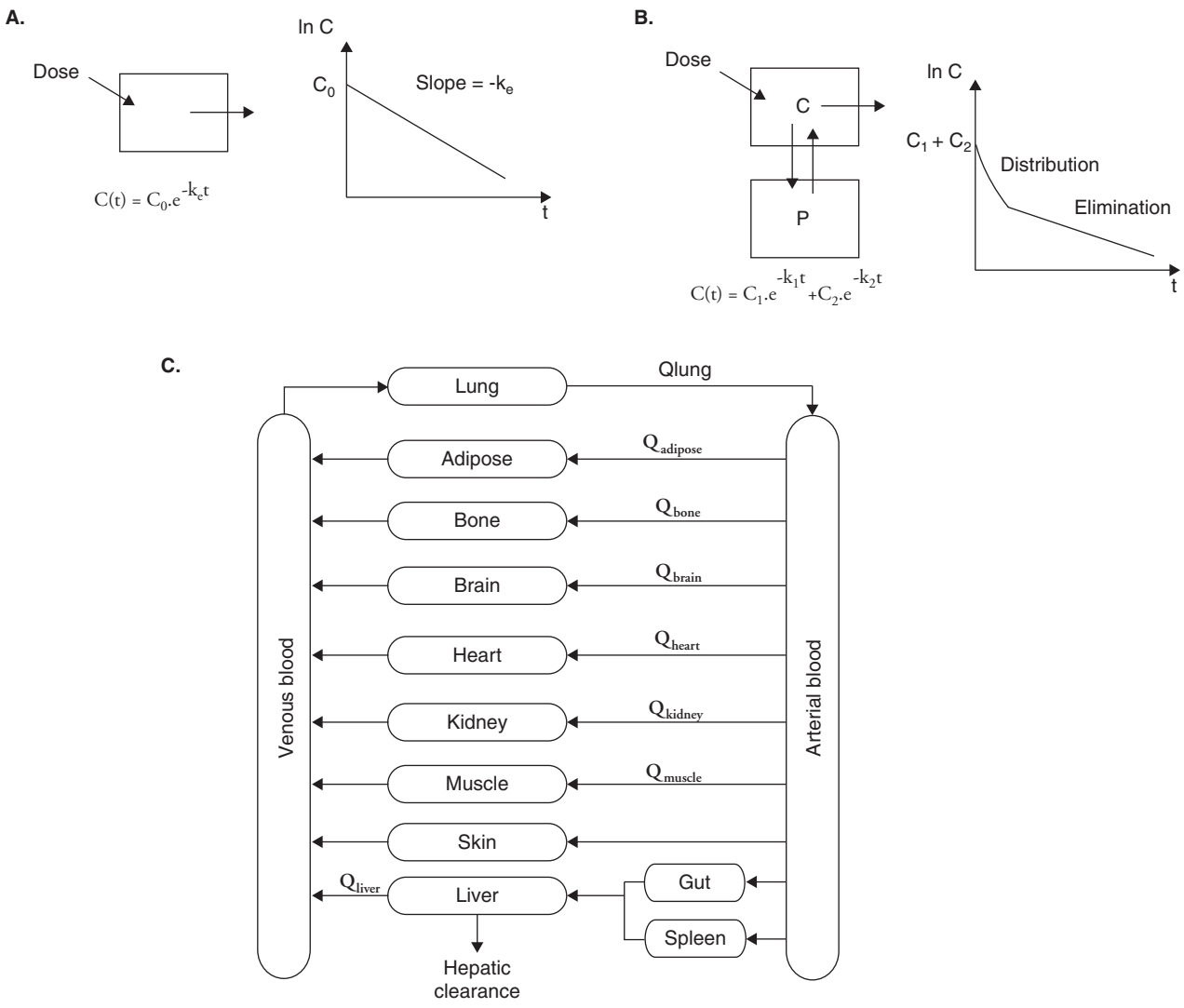

Figure 2. A. One-compartment PK model. **B.** Two-compartment PK model, which describes the PK with a sum of two exponential terms. **C.** A schematic diagram of a PBPK model. The whole body is segregated into compartments and connected with blood flow. **D.** Ordinary differential equations are used to describe the mass balance of a drug in each compartment.
PBPK: Physiologically-based pharmacokinetic; PK: Pharmacokinetics.

organs, which is often necessary and useful, will inevitably introduce empiricism into the model.

An important challenge in PBPK model development is finding accurate parameters. Physiologic parameters, such as physical volumes and blood flow rates, are generally compound-independent and can be found in the literature, although theses parameters are subject to inter-individual variations. Compound-specific parameters such as tissue-to-blood partition coefficients or enzyme kinetic parameters are generally more difficult to find. In the case where a well-mixed condition is assumed, the tissue-to-blood partition coefficient is used to account for the distribution of chemicals in tissues. These parameters can be found from tissue incubation experiments [20], or theoretical values can be extrapolated from the *n*-octanol:water partition coefficient, which requires the assumption of steady-state conditions [21]. For enzyme kinetic parameters, methods for predicting *in vivo* kinetic parameters from *in vitro* data have been proposed [22]. However, it is more common to have at least some of the parameters optimized by fitting the PBPK model to a drug concentration data [23]. Another important issue in PBPK modeling is that often differences of *in vivo* physiology between species is not considered. Also, PK can be affected by disease conditions, which are often not considered. Introduction of statistical approaches, such as population (nonlinear mixed effects) modeling and Bayesian hierarchical models, has allowed researchers to develop improved models in spite of the limited availability of experimental data, and also to account for variability (inter- and intra-individual and subject covariates) in such systems [24,25].

2.3 PD model

PD refers to the time course of a pharmacological effect at a given drug concentration. The basis of PD is that the pharmacological effect of a drug is a function of drug concentration. Depending on the drug's mechanism of action, PD models can be roughly divided into several subgroups, some of which are briefly introduced here. Comprehensive and exhaustive classification of PD models is beyond the scope of this article, and we recommend readers to other excellent reviews [26]. The simplest form of a PD model assumes a direct, linear relationship:

$$E = E_0 + S \cdot C \tag{1}$$

where E is the drug effect, S is the slope and C is the drug concentration. Obviously, this model unrealistically predicts that the drug effect will increase indefinitely with an increase of drug concentration. However, it has been applied to several types of drugs [27,28]. Another well-known form of a PD model is an E_{max} model (**Figure 3A**), which is expressed as follows.

$$E = \frac{E_{max} \cdot C}{EC_{50} + C} + E_0 \tag{2}$$

E_{max} is the maximum drug effect and EC_{50} is a concentration at which 50% of the maximum drug effect is seen. This model can define the maximum effect and, therefore, is more likely to be valid in all concentration ranges. It has been used to describe the effect of antimicrobial drugs [29] and the neutropenia resulting from a chemotherapeutic agent, paclitaxel [30]. Although the E_{max} model is widely used and useful, it can only describe the reversible effect of a drug, and does not assume any time-dependent processes. In other words, a drug effect will immediately decrease with a decrease in drug concentration. However, many drugs, for example oncology drugs, irreversibly affect cells, and the effect is often shown in time-delayed manner [31]. The first attempt to model the irreversible, time-dependent effect of a chemotherapeutic agent by using the indirect response model was done by Jusko, who modeled the effect of phase-nonspecific drug on tumor cells [31]. In this model, the irreversible effect of a drug can be modeled with a cell proliferation model, where the rate of cell death is a function of drug concentration (**Figure 3B**):

$$\frac{dn}{dt} = g(n) - f(c) \cdot n \tag{3}$$

$$f(c) = \frac{E_{max} \cdot C}{EC_{50} + C} \tag{4}$$

where n is a cell number, g(n) is a proliferation rate of cells in the absence of the drugs and f(c) describes the effect of the drugs as a function of drug concentration. This concept has evolved into several different PD models with more complex functions [32].

2.4 Integrated PK-PD modeling

A PBPK model yields predicted concentration–time profiles at specific sites, so it can be coupled with a PD model, which can predict the drug's effect at the site of exposure [4,14]. By combining a PK model and a PD model, the time-dependent pharmacological effect can be predicted from a given dose (**Figure 3C**). The integrated PK-PD model is a dynamic model, because a concentration profile from the PK model is fed into the PD model as a time-dependent variable.

There are several review articles summarizing the concept of integrated PK-PD modeling approach [4,11,12,14,15,33-35]. Here, we introduce a few examples of integrating a PBPK model with a PD model. Recently, a PBPK/PD model was developed to simulate the effect of carbaryl, an *N*-methylcarbamate, where a Bayesian approach was used for estimation of parameters of the PBPK and PD models [36]. In a series of study by Timchalk *et al.,* PBPK/PD models for organophosphorus insecticides chlorpyrifos (CPF) and diazinon (DZN) have been developed [37-39]. PBPK/PD models for each insecticide were constructed; metabolic interactions (CYP450, carboxylesterase, butyrylcholinesterase and acetylcholinesterase) between the two were evaluated by using a binary model for CPF, DZN and their metabolites. In a

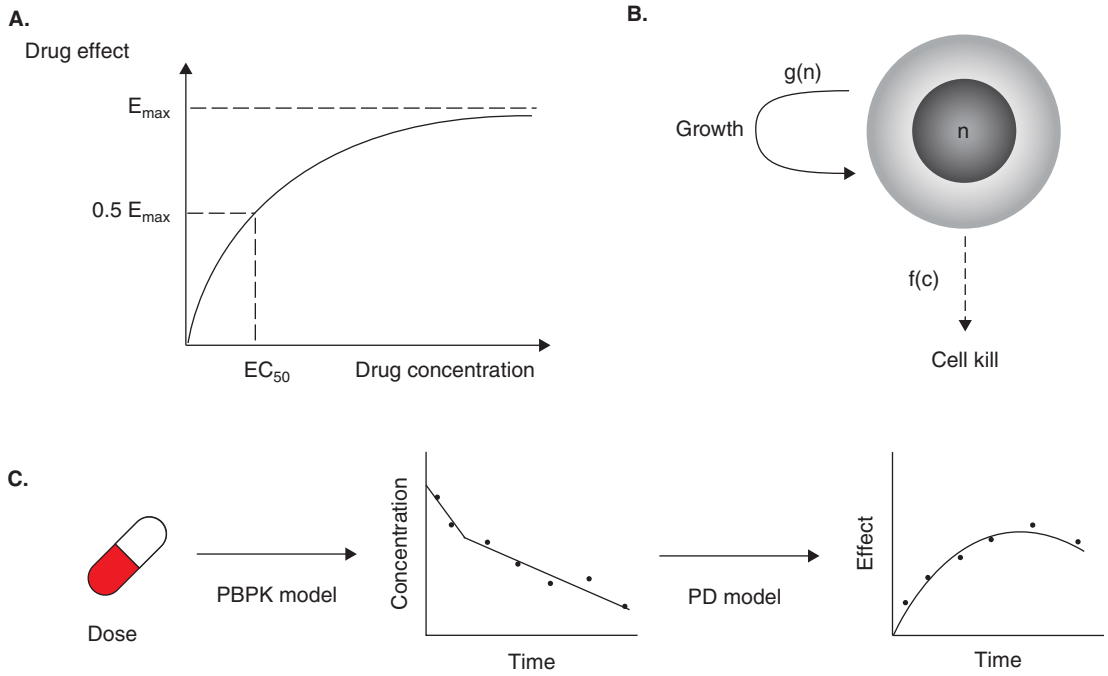

Figure 3. A. An E_{max} PD model describes the effect of a drug based on the drug concentration. **B.** An irreversible, cell death PD model describes the cell death kinetics. **C.** Integration of a PBPK model and a PD model.
PBPK: Physiologically-based pharmacokinetic; PD: Pharmacodynamic.

previous study by the authors of this paper, a PK-PD model was developed to predict the tumor growth kinetics after administration of chemotherapeutic agents in rats [40]. A PBPK model describing the fate of UFT (tegafur in combination with uracil) in rats was developed and fitted to literature data. A PD model was developed to simulate the growth of tumor in the presence of 5-fluorouracil (5-FU), which is the active metabolite of tegafur. The two models were combined to describe the growth of tumor in rats following UFT administration.

The integrated PK-PD model is believed to have an important role in expediting the drug discovery process [11]. However, as mentioned earlier, identification of mechanism-based parameters can be challenging. In fact, this limitation is thought to be a primary obstacle to a wider application of PK-PD models [7]. The parameterization of mechanism-based models should take into account drug- and system-specific properties to warrant predictive performance across species. In addition, sufficient amount of human data should be available to ensure model validity. Another limitation of a PK-PD modeling approach is that it is not possible to predict any outcome that results from a mechanism that is not included in the model. While a PK-PD modeling approach can be used to gain an important insight into the action of a drug in the human body, an experimental platform is always desirable. The *in vivo* counterpart of a PK-PD model is a human clinical test or an animal study, which can be expensive, time consuming and pose ethical issues. Conventional *in vitro* platforms cannot reproduce multi-organ interactions and feedback mechanisms that are present in *in vivo* situations. If an *in vitro* counterpart of a PK-PD model can be developed, it would be able to serve as a link between an *in silico* PK-PD model and *in vivo* animal and human tests. A hypothesis in a PK-PD model can be tested *in vitro,* which will ultimately reduce the need for animal or human tests.

3. Microscale devices for an *in vitro* PK-PD model

The use of microfabrication technology, which was originally developed in the semiconductor industry, has been extended to other research areas, such as microelectromechanical systems or microfluidics technology. In particular, integration of microfabrication and cell culture techniques in biology has resulted in a novel 'cells-on-a-chip' technology [9,41,42]. Combining cell culture and microfabrication technology confers new advantages to the previous (macroscale) cell culture system. First, structures with a micrometer resolution can be precisely fabricated, mimicking the typical length scale in biological systems. Cells that are cultured in an environment closely mimicking their native environment are more likely to exhibit their authentic functions than cells cultured in flasks or 96-well plates due to more realistic environmental parameters, such as flow rates, shear and liquid:cell ratio. For example, the flow around the cells and the resulting shear

are known to affect cell function [43]. In microscale devices, those parameters can be tuned precisely to match the fluidic environment of the native tissue. Furthermore, microscale devices allow the transport of nutrients and oxygen to be controlled for optimal cell function. For example, some cell types exhibit better cell function when oxygen tension is low rather than in an oxygen-saturated environment [44]. Second, miniaturization enables high-throughput implementation, which can save novel reagents and scarce tissue samples. In particular, microfluidics can dramatically enhance efficiency by creating multiple conditions simultaneously. For example, a concentration gradient can be easily formed by utilizing a laminar flow in a microchannel, exposing cells to a series of conditions in 'one shot', rather than preparing different conditions separately [45]. Last, microfabrication technology allows researchers to create multiple components and interconnect them. This feature has an important implication in that several components (representing different organs) can be interconnected to form an 'artificial body', also known as a 'body-on-a-chip', providing an *in vitro* platform for a PK-PD model [46,47].

A microfluidic device developed by Ma *et al.* illustrates an interesting example of these advantages, where a sol-gel human liver microsome (HLM) bioreactor was coupled with a cell culture chamber array (**Figure 4**) [48]. This device has a three-layer structure, a top layer for fluidic channels, a middle quartz layer with microwells for HLM and a bottom polydimethylsiloxane (PDMS) layer for cell culture chambers. A drug is introduced into the top layer and diffuses into the HLM. Metabolites generated from the HLM then diffuse down to the cell culture chamber, where the response of cells is monitored. In another study, a hepatocyte-bioreactor coupled with a microscale cell culture device was used to assess the hepato-activated transformation of cyclophosphamide [49]. Primary hepatocytes were cultured in a perfusion bioreactor, connected to a cell culture chamber. Lee *et al.* developed an array of sol-gel encapsulated P450 enzymes (denoted by the authors as MetaChip) [50]. A prodrug is added onto the MetaChip, generating metabolites, and cytotoxicity is assessed by overlapping with another layer of target cells (DataChip). Drugs showed toxicity profiles similar to a solution control, which verified that the sol-gel arrays could produce comparable toxicity assay results in a high-throughput manner. These examples demonstrate the effort to observe metabolism-dependent toxicity profiles of drugs *in vitro,* but one shortcoming is that these approaches fail to address the quantitative relationship between different organ compartments and the dynamics of drug exposure.

In an effort to conserve the quantitative relationship between different organs, Vozzi *et al.* developed a multicompartment bioreactor, where multiple cell types are cultured in separate chambers [51]. In this study, an effort to conserve the ratios of kinetic, metabolic and volumetric parameters between compartments was made by using an allometric scaling law to scale down the human body to the microscale device. The crosstalk between murine hepatocytes and HUVEC was investigated. An interesting observation was made that albumin and urea synthesis was enhanced when the two cell lines were co-cultured in the same device without direct, physical contact. A multi-channel 3D microfluidic cell culture system was developed by Zhang *et al.* for the purpose of building a 'human-on-a-chip', with four different cell types cultured in a single device [52]. An interesting approach was made by the authors to maintain 'organ-specificity', while still allowing communication between the compartments, by utilizing gelatin microspheres to entrap and release TGF-β1 locally. TGF-β1 enhances lung cell function, but adversely affects liver cells, and so the release of TGF-β1 was confined within the lung compartment.

A μCCA was originally developed as a physical representation of a PBPK model. Multiple compartments are fabricated on a silicon chip (30 × 30 mm), which are interconnected with fluidic circuits mimicking blood circulation [10]. Different cell types representing organs are cultured in the corresponding compartments (liver, lung, tumor, etc.). Taking advantage of microfluidics, the flow rates and residence times in each compartment are set at values specific to the corresponding organs, mimicking the dynamics of drug exposure to the organs. As a proof-of-concept study, a four-chamber μCCA to probe naphthalene toxicity was designed and fabricated containing the lung, liver, fat and other tissue chambers [53]. Naphthalene was added to the re-circulating medium, and the response of cell lines was observed by monitoring glutathione (GSH) levels. While being re-circulated in a μCCA, naphthalene is converted to active metabolites by the liver cells in the liver chamber, which then circulate to the lung chamber, depleting GSH in the lung cells. This study demonstrated for the first time that a μCCA can be used to test metabolism-dependent toxicity of a drug, by mimicking organ interactions in the body. In a subsequent paper, additional differentiated 3T3-L1 adipocytes were cultured in a fat chamber, mimicking bioaccumulation [54].

In a more recent study, a μCCA was fabricated to study multidrug resistant (MDR) cancer [55]. In a four-chamber μCCA, the liver, bone marrow, uterine cancer and an MDR variant of uterine cancer were cultured, and treated with various combinations of a chemotherapeutic agent and modulators. A significant improvement was made in device operation, which made it possible to operate the device for up to 72 h with medium re-circulation. A PBPK model of the μCCA was developed and compared with a PBPK model of human body, which would allow a direct extrapolation of drug doses and AUC values from the μCCA to the human body.

Although these studies successfully demonstrated the concept of a 'PBPK model on a chip', cell lines cultured in the μCCA are far from the authentic representation of real organs, as these are immortalized cell lines, which can be significantly different from primary cell cultures. Recently, cryopreserved human hepatocytes were cultured in a liver chamber of a

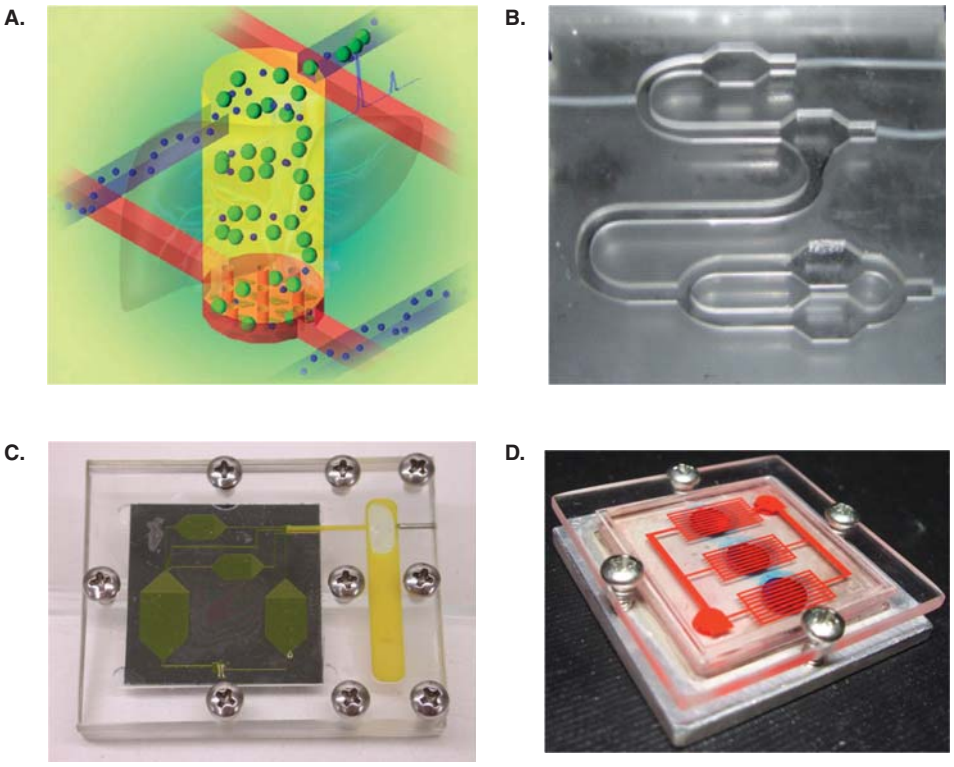

Figure 4. A. A three-layer microfluidic device for examining the metabolism-dependent toxicity of drugs [48]. **B.** An MCB cell culture chamber [51]. **C.** A four-chamber, silicon μCCA encapsulated in a plastic housing with a bubble trapping chamber. **D.** A three-chamber PDMS μCCA for hydrogel-encapsulated cell cultures. Hydrogel-encapsulated cells are cultured inside round-shaped chambers and cell culture medium is provided through fluidic channels, covering the hydrogel matrix on top.
Figure 4A was reproduced with permission from the Royal Society of Chemistry and Figure 4B was reproduced with permission from Mary Ann Liebert, Inc.
MCB: Multicompartmental bioreactor; PDMS: Polydimethylsiloxane; μCCA: Microscale cell culture analog.

μCCA device, and the hepatic clearance rates of six marketed model compounds were compared with clearance rates *in vivo* and from static cultures [56]. In this study, clearance data obtained from the device were scaled-up directly to predict human hepatic clearance. Another limitation of a μCCA was that cells were cultured in 2D monolayer, whereas real organs are made of many different cell types, with complex 3D structures. To overcome this shortcoming, we have incorporated hydrogel-encapsulated cells in a μCCA [57]. In this study, a three-chamber μCCA was used to culture colon tumor, liver and myeloblast cell line. The three cell lines were encapsulated in Matrigel^{TM} or alginate, and cultured in separate chambers of a μCCA. Tegafur, a drug for colon cancer, was chosen as a model drug, which is not toxic to cells itself but becomes toxic after being converted to 5-FU, mainly in the liver. Consistent with the known mechanisms of action, the toxicity of tegafur was observed, but only in the presence of liver cells. In a more recent study, microfluidic 3-D hydrogel cell culture was combined with a mathematical PK-PD model to test the effect of a chemotherapeutic agent, 5-FU, combined with a modulator, uracil [58]. Model-based analysis allowed improved insight into the drug's action. Interestingly, cells cultured under the microfluidic environment showed different responses to the drugs compared to the same cells under static conditions, implying that current *in vitro* tests may not adequately reflect the *in vivo* situation where cells are exposed to continuous flow.

4. Microfabricated *in vitro* systems for reproducing PK (artificial organs)

In conventional cell culture techniques, cells are cultured in a flask or a culture dish, and such a macroscale environment does not provide a milieu similar to the native organ. Consequently, in many cases, cells that are cultured *in vitro* would not be fully functional and do not provide an adequate platform for an examination of specific organ functions. Recently, advances in microfabrication technology have enabled creation of experimental conditions for cultured cells that mimic physiological, *in vivo* environment, which is not possible in conventional systems [42]. Ultimately, such devices will be useful in an approach to develop an *in vitro* platform of a PK-PD model, because the faithful reproduction of an organ's function is essential for this purpose.

4.1 Liver (biotransformation)

The liver is the most important organ in terms of biotransformation responsible for the majority of detoxification and bioactivation reactions that drugs undergo. It is thought that the liver is strategically located between the digestive organs and the other parts of the body [59]. The metabolism in the liver is responsible not only for detoxification or bioactivation, but also for drug–drug interaction. Given the importance of the liver, there have been many efforts to develop *in vitro* platforms that can reproduce the metabolic profile of the human liver [59,60]. Furthermore, integration of diverse areas such as microfabrication, cell culture, microfluidics and reactor design is believed to present great opportunities for achieving new systems that are able to reproduce the hepatic function more closely [61,62].

An interesting aspect about the liver is that a gradient of oxygen concentration exists, and it has been known that the liver function is modulated on the basis of local oxygen concentration [63]. For example, the inlet and outlet sides of a functional liver subunit, acinus, perform different metabolic functions. At the oxygen-rich periportal side, near the inlet, biosynthesis of substances such as urea, albumin and glucose, and detoxification by GSH conjugation are predominant. On the other hand, in perivenous side, near the outlet, storage of glycogen and detoxification by CYP enzymes are more active. This observation has an important implication that current *in vitro* culture methods, which usually try to maximize oxygen transport, may not be optimal for certain metabolic activities of the liver [64]. The laminar flow inside a microfluidic device allows one to create a defined concentration gradient of molecules, by controlling the flow rate and the geometry of channels [45]. Taking advantage of this feature, a microfluidic flat-bed bioreactor was developed where rat hepatocytes were cultured with supporting non-parenchymal cells [65]. A gradient of oxygen concentration was created across the reactor. A differential CYP activity along the gradient was observed, similar to the liver.

There have been many attempts to culture hepatocytes in microfluidic devices. One of the earliest attempts was reported in a series of papers by Leclerc *et al.* who cultured cells in 3D by stacking multiple PDMS layers, where cells showed enhanced metabolic profiles [66-68]. Another notable approach was taken by Griffith and co-workers who developed a microfluidic bioreactor with perfused 3D liver cell culture. Hepatocytes were cultured inside through holes in a silicon wafer, with the flow providing nutrients to the cell matrix [69]. Interestingly, pre-aggregation of cells into a spheroid-like form prior to seeding into the device improved the liver-specific cell behavior, such as albumin secretion and urea synthesis [70]. Gene expression profiles and biochemical activity of the hepatocytes in the device were shown to have superior metabolic profiles than the same cells cultured as collagen sandwich or with Matrigel in a static environment [71]. With a combination of a mathematical model, appropriate operating parameters were predicted for culturing primary hepatocytes, which remained healthy after seven days of culture [72].

One method widely used to enhance the metabolic profile of cultured liver cells is to co-culture with supporting cells, such as epithelial, Kupffer and fibroblasts [73]. This has an obvious biological implication, because hepatocytes in the liver are surrounded by other cell types and the extracellular matrix. Micropatterning technology allows one to organize cells in a defined geometry with a micrometer resolution, rather than culturing multiple cell types in a random mixture. Utilizing this technique, it was possible to seed hepatocytes and fibroblasts with a control over the size and the spacing of 'cell islands', and find optimal geometric parameters [74].

4.2 Kidney (excretion)

One of the most promising areas that microfabrication technology can contribute to is the kidney, or renal assist devices. Patients with renal failure have been treated with *ex vivo* device utilizing hemodialysis membranes [75]. The traditional method has been the use of hollow-fiber dialyzers, using cellulose as the membrane material [76]. The periodic dialysis treatment causes sudden changes in their blood chemistry and fluid volume due to the non-physiological flow rate in the artificial renal devices [77]. The ability of microfabricated systems to create a controlled laminar flow offers a great opportunity for more physiologically-realistic renal devices. An interesting example was demonstrated by Leonard *et al.* who utilized microfluidics to achieve membraneless dialysis [78,79]. At a low Reynolds number, two miscible flows can be introduced in parallel without significant convective mixing, with diffusion being the dominant transport phenomenon. Because small molecules diffuse faster than larger molecules, it is possible to separate small size solutes from macromolecules in the blood, mimicking the dialysis process. In another example by Nissenson *et al.,* an artificial renal device, termed as a 'human nephron filter' was developed [80]. In another approach, microfabrication was utilized to improve the transport process by tweaking the geometry at nanometer length scale [81].

The hemodialysis approach suffers from eventual decrease in the filtration rate due to protein deposition on the membrane. Furthermore, this approach does not reproduce the full function of kidney, for example, metabolic or endocrine function. The development of a renal device utilizing cells will be a promising solution for such shortcomings [82,83]. Another practical limitation of the conventional hemodialysis approach is the requirement of intermittent, thrice-weekly hemodialysis at the treatment center, which impacts the patient's quality of life due to travel time and dietary restrictions. This limitation, with the advent of microtechnology, has motivated the development of a wearable artificial kidney device, which has been under human pilot studies recently [84].

While the dialysis approach to artificial kidney only replicates the function of glomerulus, the human kidney and

its subunit nephron consist of a glomerulus, proximal tube and the loop of Henle, which perform together the function of removing the waste while maintain body homeostasis. Recently, Weinberg *et al.* proposed the concept of a bio-artificial 'nephron-on-a-chip', which is a microscale device mimicking the function of the three units comprising the nephron (**Table 1**) [85].

4.3 Lung (gas exchange)

The lung, the respiratory organ, is responsible for the exchange of oxygen and carbon dioxide between atmosphere and bloodstream. The actual gas exchange occurs in the extremely thin-walled subunits of the lung called alveoli, which are surrounded by pulmonary capillaries. Efficient gas exchange is ensured by the highly branched structure of the lung and the organization of alveoli, maximizing the surface area:volume ratio, which is the amount of surface area per unit volume of an object [86]. A conventional artificial lung consists of hollow fiber membranes for gas exchange, which is far from mimicking the complex geometry of a natural lung [87]. This structural organization of the lung presents an interesting challenge and opportunity in engineering, micro/nanotechnology in particular.

In the papers by Mockros and co-workers, the possibility of a microchannel artificial lung was explored. Circular or rectangular channels with various sizes and designs were analyzed mathematically [88]. An attempt was made to mimic the length scale of real capillary blood vessels (5 ~ 10 μm), while maximizing the gas transfer with a low pressure drop [89,90]. 3D arrays of blood microchannels and gas pathways were fabricated in PDMS, with the blood microchannels lined with endothelial cells to reduce coagulation [91]. These layers were stacked to create modules with up to six layers, with a large surface area:volume ratio. This result seems to be a promising achievement towards an artificial lung module. However, another challenge with an artificial lung device using micro-scale channels is that a large number of uniform channels need to be fabricated and operated in a reliable manner. From the measured gas permeance values in the device, it was calculated that about $5 \times 10^5 \sim 1 \times 10^6$ channels are required to achieve the gas exchange level comparable to the lung. Even with the recent development in microfabrication technology, further improvement is still needed to achieve this.

4.4 Gastrointestinal tract (absorption)

Oral drug uptake is one of the preferred routes of drug administration because it is simple and patient compliance is high. Drugs suited for oral administration possess physicochemical properties that allow them to withstand microorganisms, degrading enzymes and bile salts, while passing across the digestive barrier. Several *in vitro* models for intestinal drug absorption studies have been developed [92]. Among these, the Caco-2 cell model is one of the best characterized and utilized [93]. It is well suited to simulate the physical barrier of the lumen gastrointestinal tract. After differentiation for 2 weeks, the Caco-2 cells form polar, enterocyte-like epithelial cell layers with tight junctions and microvilli.

Epithelial cell layers composed of differentiated Caco-2 cells facilitate several routes of molecular transport. Lipophilic molecules enter the cell's membranes and diffuse to the basolateral side (paracellular transport) [94]. Relatively small, hydrophilic molecules enter the cells from the apical side and diffuse through them to the basolateral side (transcellular transport) [95]. Both transport processes depend on the concentration gradient of the drug across the cell layer as well as its ability to diffuse through the unstirred water layer present at the apical cell layer surface and the ability to diffuse through the cells and membranes. Other possible transport processes facilitated by Caco-2 cell layers are transcytosis via vesicles, active carrier-mediated transport and efflux transport through which the drug is returned to the intestinal lumen but causes another ion or molecule to move against its electrochemical gradient [96].

To represent the intestinal lumen more realistically, other cell types have been included with the Caco-2 cell model [97]. For example, HT-29MTX cells produce mucous that characterizes the human intestine, and *in vivo,* mucous producing cells make up about 10 – 25% of the cell layer. Co-culturing the two cell types at this ratio is thought to result in mucous production that plays a role in the bioavailability of iron [97]. μCCAs of Caco-2 cell models can be combined with hepatocyte models and provide a platform that can be used to simulate first pass metabolism. Static Caco-2 cell models have been used in co-culture with hepatocytes [98,99], but the amount of medium needed was rather large and could not be re-circulated without a fluidic system. Incorporating the model into a μCCA system with a downstream liver compartment provides the opportunity to re-circulate medium and to achieve realistic residence times [100,101]. A practical challenge in such systems is the need to balance apical and basolateral fluid flow so that no significant mechanical forces are exerted on the Caco-2 cell layer that is typically cultured on a porous membrane. When designed with drug residence times close to those observed *in vivo,* such cell culture analog systems simulated realistically the absorption of acetaminophen through the intestinal epithelium and to the liver; Liver toxicity was observed in a dose-dependent manner similar to animal studies [101].

Even though advances have been made in the development of *in vitro* systems of the digestive tract, no single system developed so far is capable of simulating the complex digestive process in its entirety. For example, the Caco-2 cell's expression of uptake transporters is lower than that of *in vivo.* The system also lacks the biochemical barrier function due to the microflora present in the intestinal lumen, the whole range of metabolic enzymes and other constituents such as bile salts. Nevertheless, data obtained with Caco-2 *in vitro* models together with data obtained with PBPK models may be useful in determining more realistic starting doses for *in vivo* tests of newly developed drugs.

Table 1. Comparison of microscale devices and conventional technologies for mimicking the functions of specific organs.

	Systems		Advantages	Limitations	Relevant parameters	Values in device	Reported values in human [125]
Liver	Conventional systems	Human liver microsomes	Relatively simple and easy to use	Unbalanced enzyme composition	Shear stress/residence time		0.1 ~ 0.5 dyne/cm^2 70 sec [126,127]
		Primary hepatocytes	Good reproduction of human liver	Rapid loss of enzyme activity			
	Microscale systems	A flat-bed microreactor [65,128]	Liver zonation with oxygen concentration gradient	Short-term experiment (~ 3 days) and 2D monolayer cell culture		1.25 ~ 7.5 dyne/cm^2 2.31 ~ 12.8 sec	
		Microfluidic PDMS bioreactor [66-68]	Enhanced oxygen delivery	Hepatoma (incomplete metabolic profile)		0.03 ~ 0.25 dyne/cm^2 0.77 ~ 1.54 sec	
		Microfluidic, 3D cell culture device [69-72]	3D cell organization and scalable device for high-throughput implementation	No supporting cell types		0.05 ~ 0. 35 dyne/cm^2 2.2 ~ 4.4 sec	
Kidney	Conventional systems	Hemodialysis membrane/ hollow-fiber dialyzers	Diffusive transport of small waste molecules, while retaining higher MM compounds	Undesirable periodic treatment/ membrane fouling	Shear stress/residence time		
	Microscale systems	Renal cell microchip [83]	High cell density/viability/ proliferation	No waste removal function		0.02 ~ 0.06 dyne/cm^2 72 ~ 180 sec	0.03 ~ 0.4 dyne/cm^2 (distal renal tubule) 13.5 sec [83]
		Microfluidic device with primary kidney cells [129]	Multi-layer with realistic shear stress	No waste removal function		1 dyne/cm^2 N/A	0.1 ~ 20 dyne/cm^2 (renal tubule) 13.5 sec [130-132]
		Membraneless transport coupled with sheath dialysis [78,79]	Can avoid hemocompatibility issue	No metabolic/ endocrine functions		N/A	
Lung	Conventional systems	Hollow-fiber membranes	Blood is transversely mixed to maximize transport	Low surface: volume ratio, biocompatibility	Surface:volume ratio/total surface area	30 cm^{-1}/2 ~ 4 m^2 [91,133]	300 cm^{-1}/ 100m^2 [86,134]
	Microscale systems	3D PDMS arrays of blood/ gas pathways lined with endothelial cells [91]	Improved surface area	Fabrication of a large number of channels		1000 cm^{-1}/2.5 ~ 5 m^2	

Parameters in the table were either directly taken or calculated from the cited references.
GI: Gastrointestinal; MM: Molecular mass; N/A: Not available due to insufficient information from the literature; PDMS: Polydimethylsiloxane.

Table 1. Comparison of microscale devices and conventional technologies for mimicking the functions of specific organs (continued).

	Systems		Advantages	Limitations	Relevant parameters	Values in device	Reported values in human [125]
GI tract	Conventional systems	Transwell co-culture model [98,99]	Co-culture of GI-tract and liver cells	Static cell culture, no re-circulation of medium	Surface:volume ratio	1.12 cm^{-1}	1000 cm^{-1} [98,135]
	Microscale systems	Perfusion system with Caco-2 cell on membrane [136]	Fluid flow is present	No medium re-circulation		~ 8 cm^{-1}	
		Fluidic chamber with transwell insert [100,101]	Physiological fluid flow rates in liver compartment	Multi-organ interaction by medium re-circulation		20 cm^{-1}	
Microvasculature	Conventional systems	Boyden chamber/ transwell [137]	Migration assays are possible	Static, flat surface does not reflect the geometry of blood vessels	Shear stress	No shear stress	~ 40 dyne/cm^2 [138]
	Microscale systems	Parallel plate flow chamber/modified Boyden chamber [139-141]	Physiological fluid flow is present	Flat surface does not reflect the geometry of blood vessels		~ 24 dyne/cm^2	
		Collagen tubes populated with endothelial cells [105]	Confluent vessels with round geometry achieved	Single channel, no complex vessel network		~ 10 dyne/cm^2	
		Microfabricated vessels populated with endothelial cells [107]	Complex vessel networks are achievable	No migration assays possible		N/A	

Parameters in the table were either directly taken or calculated from the cited references.
GI: Gastrointestinal; MM: Molecular mass; N/A: Not available due to insufficient information from the literature; PDMS: Polydimethylsiloxane.

4.5 Vascular network (gas and nutrient transport)

The endothelial lining of the microvasculature is another barrier tissue for which efforts have been made to develop *in vitro* μCCAs. In conventional models of the microvascular endothelium, HUVECs or human dermal microvascular endothelial cells are cultured on porous membranes in stationary chambers (Boyden chambers) or in parallel plate flow chambers that provide medium flow [102-104]. These systems can be used to assess the permeability of the endothelial lining towards drugs and cancer cells as well as to record changes in permeability in response to vasoactive agents.

Several groups have developed μCCAs of the endothelial lining. Collagen tubes that are crosslinked around needles that were subsequently removed provide a round, concave lumen ranging in size from 100 to 200 μm in diameter. These tubes have been used to form hollow endothelial vessels [105]. While this fabrication method is capable of replicating the *in vivo* geometry of the surface on which endothelial cells grow, it is limited to relatively large lumen diameters and single un-branched tube geometries. To achieve smaller lumen diameters, microfabrication methods have been adapted to fabricate tubes with rectangular, trapezoidal or circular cross-sectional profiles with diameters as small as 50 μm in biocompatible, polymeric materials [106,107].

The most important criterion for a successfully engineered *in vitro* microvasculature is the presence of the endothelial barrier function. HUVECs establish a barrier function within days when seeded at a sufficiently high density. The establishment of the barrier function in 3D models (100 – 200 μm) can be promoted by rotating the tubes during cell culture [105]. If the scaffold matrix consists of a material into which molecules can diffuse, such as collagen, the barrier function can be measured by introducing fluorescently labeled proteins of various sizes into the endothelial tube and measuring the amount of protein that diffuses into the matrix [106]. Microfabricated tubes with small diameters bear the challenge of seeding endothelial cells at a density high enough to establish adherence junctions, because their surface area:volume ratio becomes very high. Hence, very concentrated cell solutions must be used. Microscale cell culture models of the microvasculature can be used to study circulatory diseases such as cancer metastasis, immune-mediated diseases and inflammation.

5. Conclusion

In this review article, we introduced briefly the concept of PK-PD modeling and explored the experimental research efforts directed towards reproducing the PK-PD of drugs by mimicking the physiology of human body or specific organs. Recent, rapid developments in combining microfabrication technology with cell culture techniques have yielded novel devices with functions that closely mimic natural organs. In particular, a μCCA enables characterization of drug effects in a quantitative manner, which has not been possible in conventional multi-well plate systems. Although there are several remaining challenges in achieving a true, 'human-body-on-a-chip', recent progress shows a great promise for devices that can complement animal and human studies.

6. Expert opinion

PK-PD modeling is an established, but still developing field since its emergence several decades ago. This technique has diverse applications including toxicity assessment, dose optimization, cross-species extrapolation and characterization of drug–drug interactions [14,108]. Further development in mechanism-based PK-PD modeling is required to enhance its predictive performance in drug development and overcome current limitations in the extrapolation from *in vitro* to *in vivo* drug properties, within and across species. Some of these limitations can be addressed by integration of microscale technology concepts to PK-PD models.

The area of microscale technology has been growing rapidly, offering researchers a new ability to investigate biological problems with an unprecedented ability to control cellular environment. It has had a significant impact in various areas, including tissue engineering, systems biology and high-throughput drug screening [8,109,110]. We believe that another area with a great potential is PK-PD modeling. Microscale technology enables the development of a physical realization of a mathematical model, and allows one to make direct comparisons between the *in vitro* and *in silico* platforms. By having a physical, *in vitro* counterpart of a mathematical model, various hypotheses can be tested directly, which are difficult to be tested in animals or humans. For example, various drug combinations ratios and dosing schedules can be easily tested, while exploring the whole parameter space in a large combinatorial trial in animals or humans would be a daunting task. Furthermore, the combination of a μCCA and PK-PD modeling can provide an improved basis for animal to human extrapolation, with improvements in culturing animal-originated cells or human-originated cells. Such advantages will allow shifting the assessment of drug effects in humans to an early stage of drug development. In addition, it enables the identification of potentially toxic compounds as well as of mechanisms underlying toxicity.

Although this possibility has been recognized only recently, several novel examples of utilizing microscale technology to reproduce the complex action of drugs in the human body are emerging [111]. The μCCA devices first developed at Cornell and adapted by others have demonstrated that the effect of drugs arising from multi-organ interaction can be observed experimentally [53,55,57]. Several microscale or microfluidic systems have been developed to examine the metabolism-dependent effect of drugs [48,50]. Significant amount of progress has been achieved in terms of reproducing the functions of specific organs *in vitro,* such as the liver, lung and kidney [62,75,77,81]. Obviously, the examples illustrated in

this review article are still far from a complete reproduction of PK-PD model, and only are a partial representation of what happens in the human body. Nevertheless, even modest improvements (for example, from 10 to 20% success rate in clinical trials) would have a significant impact on the cost of drug development.

Microscale/microfluidic devices have their own limitations, and many issues remain to be solved before they can be more widely accepted [112]. For example, various new materials have been used in microfluidic devices, but the biocompatibility of these materials needs to be characterized better. The exact effect of a microfluidic environment on a cell's behavior needs a deeper investigation. Furthermore, fabrication, assembly and operation of microfluidic devices need to be standardized and made more 'user-friendly' for high-impact applications [113,114]. For example, air bubble formation in a microfluidic system is an important issue that needs to be characterized better [115,116]. Another important issue in microscale systems is detection and analysis, as miniaturization renders assays more difficult than macroscale systems where a larger volume of fluid is available. Typical sample volumes in microfluidic systems (in nanoliter range) make it difficult to analyze samples with conventional analytical equipments. Moreover, often the closed nature of such systems requires non-invasive detection. Improving or modifying current analytical techniques to adapt to new microfluidic devices has been an active research area [117-121]. Although fluorescence optical detection has been the main technique for non-invasive detection, utilizing electrical signals or cellular impedance has also been proposed, which might be more suitable for high-throughput applications [122-124].

In conclusion, although still at an infant stage, combining microfluidic systems and the mathematical modeling approach holds a great promise, both from the fundamental scientific and practical viewpoints for improved methods of drug development and determination of chemical toxicity.

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