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Body-on-a chip: Using microfluidic systems to predict human responses to drugs*

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Abstract: Using an in vitro platform technology that combines microfabricated devices with cell culture, we seek to understand the response of the human body to pharmaceuticals and combinations of pharmaceuticals. Computer models of the human body guide the design of in vitro systems we call micro cell culture analogs (μCCAs) or "body-on-a-chip" devices. A μCCA device is a physical representation of a physiologically based pharmacokinetic (PBPK) model and contains mammalian cells cultured in interconnected microchambers to represent key organs linked through a circulatory system. μCCAs can provide inexpensive means for realistic, accurate, and rapid-throughput toxicological studies that do not require experimenting with animals and reveal toxic effects that can result from interactions between organs. As the natural length scale in biological systems is on the order of 10–100 μm, operating on the microscale allows us to mimic physiological relationships more accurately. We summarize proof-of-concept experiments using mixtures of drugs to treat multidrug-resistant (MDR) cancer and colon cancer. We discuss the extension of the μCCA concept to systems that connect barrier tissues with systemic circulation. Examples with models of the gastrointestinal (GI) tract are provided.

Keywords: barrier tissues; gastrointestinal tract; high-throughput screening; micro cell culture analogs; microfluidic cell culture model.

INTRODUCTION

We believe that by combining microfabrication, microfluidics, and cell culture we can develop surrogates of animals and humans that can improve both the drug development process and the testing of chemicals for toxicity. The purpose of this paper is to review one class of such surrogates that are based on mathematical models of animal and human physiology and of the underlying mechanisms of drug action or toxicity.

The drug development process is inherently inefficient with strong reliance on animal trials and subsequent human clinical trials. Animal studies are costly, time-consuming, and often raise ethical issues. At the same time, animal metabolism and cellular response to chemical signals can differ considerably from those in humans. This is reflected by the fact that only about 10 % of drugs entering human clinical trials are approved for human consumption [1]. The number of new molecular entities approved in the United States yearly has declined from 53 in 1996 to 21 in 2008 [2], reflecting the challenges in identifying useful pharmaceuticals. Clinical trials are extremely expensive. If a method could be devised that improved success rates from 10 to 25 %, hundreds of millions of dollars could be saved.

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Animal and human studies can be augmented by in vitro systems using living cells and mathematical models of drug distribution and action. Here, we describe an approach combining in vitro and mathematical models to simulate human metabolism.

Most in vitro systems are used in the earliest stages of drug development, typically to identify drug candidates. These systems primarily rely on cells cultured in multiwell plates. Often, a single cell type is cultured in an individual well, in which the ratio of fluid to cells is large and physiologically unrealistic, and a bolus dose of the test drug is used. Such systems do not mimic mammalian physiology accurately. Drug exposure in the body is dynamic rather than static and differs quantitatively in both concentration and time. The response of multiple cell and tissue types to the drug and the resulting exchange of metabolites between tissues/organs is lost in multiwell plates. In particular, such systems cannot capture the metabolism and effects of prodrugs. Mechanical forces are important in cellular responses, and the typical multiwell plate assay is static and mechanical forces are absent. Thus, we believe that in vitro assays conducted in multiwell plates often cannot act as accurate surrogates for animal or human studies.

A number of groups have generated microfabricated microfluidic systems which, when combined with cell culture, may better emulate mammalian physiology. We have reviewed this literature recently [3]. Great progress has been made with these "cells-on-a-chip" technologies, and such systems offer increasingly more realistic mimics of mammalian systems. Here we will focus on a technology developed at Cornell, which has achieved the most realistic mimics of mammalian systems to date. The reader is referred to the review mentioned above for a broader discussion of "cells-on-a-chip" alternatives [3].

USING PHARMACOKINETICS/PHARMACODYNAMICS TO BUILD BODY-ON-A-CHIP MODELS

Physiologically based pharmacokinetic (PBPK) models divide the mammalian body into a series of interconnected compartments with each compartment representing a tissue or organ [4]. The blood flow to each compartment mimics that in the body. Depending on the organ's function in the body, the compartments are treated as chemical reactors, absorbers, or holding tanks. A mathematical model of the system can predict the time-dependent concentration of a drug and its metabolites in each compartment. These PBPK models can be coupled with pharmacodynamic (PD) models. A PD model predicts the pharmacological effect of a drug or a metabolite at the level of a particular tissue. Advances in our understanding of molecular pharmacology/toxicity can be integrated into a PD model. The combined PBPK-PD model provides potentially an explicit connection between molecular mechanisms and the adsorption, distribution, metabolism, and elimination (ADME) properties at the organism level.

PBPK-PD models can augment animal studies and provide a rational basis to understanding observations on mammalian response to various drug doses. However, PBPK-PD models are limited because they contain only mechanisms that have been inserted by the modeler. Because our knowledge of mammalian and particularly human physiology is incomplete, the PBPK-PD model will be similarly incomplete. Secondary responses to exposure to a drug, its metabolites, or other chemicals released from tissues are missing.

We have proposed an approach which combines cell cultures and PBPK-PD models, that is a physical system which is an analog to the mammalian or animal body [5]. For a microscale cell culture analog (μCCA) we construct a system of chambers interconnected by microfluidic channels to simulate blood circulation. Each chamber represents an organ or tissue. The sizes of the chambers reflect the sizes of the organs or tissues in the body and in the corresponding PBPK model. In a PBPK model, a reactive mass balance equation is written that accounts for the flux of the drug into and out of each chamber and its loss (or production) by reaction or adsorption (or desorption). This balance results in an ordinary differential equation for the parental compound and a balance for each metabolite. In the μCCA, these equations are replaced by living cells or tissue constructs. Ideally, these living cells perform both the functions that are included in the mathematical model plus other unknown functions that

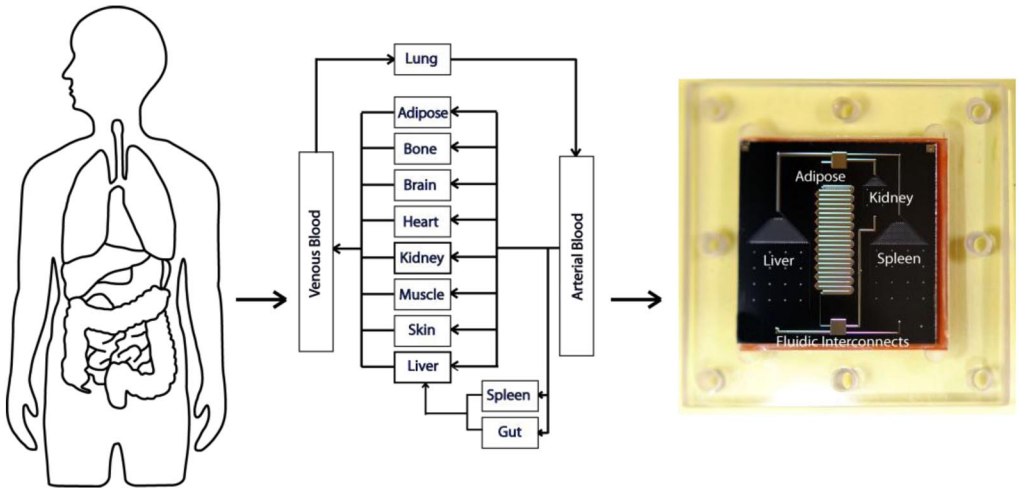

Fig. 1 Concept of μCCA development: The human body can be simulated as a series of interconnected compartments. Each organ is represented by a compartment and treated as a chemical reactor, absorber, or holding tank (depending on its function in the body). A mathematical model of the system can predict the time-dependent concentration of a drug and its metabolites in each compartment. A μCCA device (right) is a simplified version of the theoretical model that contains physical realizations of key organs on a microfluidic chip. Each organ compartment is translated into a microfluidic chamber in which shear stress and fluid residence times are equal to those seen in vivo. The compartments are connected by fluidic channels that carry and recirculate blood surrogate.

were not included due to our incomplete understanding of metabolism and physiology. Figure 1 summarizes the described concept.

The μCCA is typically fabricated in silicon using standard lithographic techniques, but it can also be fabricated in polystyrene or polydimethylsiloxane (PDMS). The resulting chip is placed in a sterile housing that is optically clear on at least one side. Several tissues are located in chambers on the chip, but nonreactive, nonadsorptive tissues can be combined in an "other tissues" compartment. The "other tissues" compartment is a vented microtube and fluid is recirculated from the chip through this tube and back into the chip using a peristalic pump. This off-chip fluid reservoir also serves the purpose of supplying oxygen, removing carbon dioxide and entrapping gas bubbles.

The residence time of a compound within each compartment controls the extent of reaction in a tissue. Control of flow to each compartment can be accomplished by altering the cross-sectional areas of each microfluidic channel leading into and out of each organ compartment. The predicted pressure drop across the channels and chambers allows us to calculate the fluid flow rate. Since the size of each compartment is known, the liquid residence time can be calculated. Thus, the residence time can be adjusted to physiological values via changes in the dimensions of the circulatory network channels. The resulting shear stress created in tissue compartments can also be calculated and should not exceed physiological values (<2 dynes/cm^2).

The first successful design has been described by Sin et al. [6] and has been termed a "body-on-a-chip" in an article in the popular press [7]. Newer designs applied to multidrug-resistant (MDR) cancer [8] and colon cancer [9] have been described.

Initial proof-of-concept experiments were done with naphthalene as a model toxicant [10,11]. These experiments were relatively short-term studies (ca. 6 h) and demonstrated that a reactive species formed in the liver could circulate to the lung, causing cell death in the lung. The experiments strongly suggested that the metabolites, 1,2-naphthoquinone and 1,2-naphthalenediol, were the reactive metabolites causing lung cell death and that the addition of a "fat" module moderated the response. Cell viability and intracellular glutathione levels were used to measure dose-dependent cell responses to naph-

thalene and naphthoquinone. This work demonstrated the feasibility of using a μCCA to address a toxicological problem.

A μCCA must meet several design criteria. The first criterion is that the ratio of one organ/tissue mass to another must be physiologically correct. If the weight of tissue A to B is 8:1 in the body, then the size of the compartments on the μCCA must accommodate a mass ratio of 8:1 of the representative cell types. The flow split must be the same as in the body. If 20 % of the cardiac output goes to an organ in the body, then 20 % of the recirculating flow must go to that compartment on the chip. As mentioned earlier, the liquid residence time in each organ/tissue compartment is important in controlling the extent of reaction. Matching the residence time in the μCCA to that in the body is a key factor in design. It is also important to recognize that mechanical forces, primarily shear stress from fluid flow, match those present in the body. Shear stress can alter gene expression and metabolic activity in the cell [12], and the geometry of the chamber must allow for a shear stress in the physiologic range for that organ/tissue type. The appropriate value varies with cell type. The four criteria above have been achieved in all systems we have developed so far.

An additional criterion is that the ratio of extracellular fluid in an organ to the cellular volume be replicated. In devices that use monolayer cell cultures [8,10], it is impractical to accomplish this since a minimal flow path (ca. 20 μm deep) is needed for robust operation. Devices with tissue-engineered constructs or high-density entrapped hydrogel cultures can more closely achieve a physiologic ratio, and the use of hydrogel-entrapped cell cultures in a μCCA has been demonstrated [9].

The most difficult criterion to meet is the use of biologically authentic tissue. This problem is universal for in vitro devices. Tissue or cells removed from the context of the body no longer behave exactly the same as in the body. Cell lines can be used to probe specific, well-posed questions where the mechanism is reasonably well understood and the amount of key enzymatic activity is present in the cell line at a level that is measurable. Primary cell lines (cryopreserved human hepatocyctes) have been used in a commercial form of the μCCA (HuRel, Beverly Hills, CA) [13]. Three-dimensional cultures, co-culture and tissue engineered systems, would all be expected to have improved activity over cell lines or even isolated single primary cells [14]. We have used 3D hydrogel cultures and have found improved specific activity [15]. While the authenticity of the response of the biological component is still a challenge, the μCCA devices we have designed can accommodate tissue-engineered constructs, tissue slices, and other 3D components.

ADVANTAGES AND CHALLENGES OF μCCAS

The μCCA differs from any other in vitro system because its design is guided by a PBPK model, which is a representation of an animal's or human's physiological structure and metabolism. We believe that unlike other in vitro systems, μCCAs should generate realistic dose dynamics for each tissue compartment. It should emulate the time-dependent change of the concentration of the parental compound or compounds and the concentration of the resulting metabolites in each chamber. Mimicking complex dose dynamics differs considerably from supplying a static concentration of a parental compound in the well of a multiwell plate. Further, the exchange of metabolites between tissues is captured in multiorgan μCCAs, but not in typically used multiwell plates.

Because the cells in μCCAs are exposed to physiological levels of hydrodynamic shear, their expression of genes and the resulting activities of the gene products are closer to the normal response than those in cells incubated under static conditions. Further, the ability to explicitly relate a PBPK-PD model of the animal to a PBPK-PD model of a μCCA allows testing of the plausibility of a proposed mechanism of action of a compound [8,16]. In a μCCA, we should know the number of cells, the amount of key enzymes related to the mechanism per cell, the flow into the chamber, the size of the chamber and hence residence time, and in some cases, the rate of release of a metabolite with reasonable precision. If our description of the proposed mechanism is complete and plausible, we should be

able to predict the dynamic response of the μCCA. If experimental and predicted values differ, the experiment suggests that our understanding of the underlying mechanism is incomplete. By an iterative study between the model and experiment, we should be able to achieve a better understanding of underlying mechanisms.

A μCCA can be used with either animal or human cells. One of the most difficult challenges in toxicology is the accurate extrapolation of responses across species. A μCCA, especially when coupled with a PBPK-PD model, should assist in extrapolation of rodent or other animal species data to a prediction of the human response.

The actual implementation of a μCCA requires solutions to several technical problems. The system developed by Sin et al. was the first example of long-term recirculation of fluid through a microscale fluidic network [6]. Most microfluidic systems have been set up for one pass flow of fluid. Recirculation of fluid is difficult because of the potential for the medium to evaporate, which would result in an increased osmolarity. This factor, as well as the accumulation of small gas bubbles, could be inhibitory to the cells. Bubbles can block fluid flow in a channel, and if they pass over cells, they can create force fields that damage or dislodge cells. The external other tissues compartment assists in bubble removal, but greater reliability can be achieved by the addition of a bubble trap, particularly for experiments that take several days to complete [17].

Another area of potential difficulty is the measurement of cell/tissue conditions in μCCAs due to the small cell number and the lack of accessibility of the recirculating fluid to removal for measurement purposes. We have used optical measurements as the primary source to probe the system, although electrical signals or cell impedance can be used (see ref. [3] for a summary). Optical measurements of cell viability have been accomplished with a variety of live/dead dyes (see refs. [6,8,9] for examples). Optical measurements have also been made of glutathione levels [6,10,11], uptake of a drug [8], and expression levels of protein using green fluorescent protein (GFP) [18]. In other systems, electrical measurements have been used [3,19–21] in combination with optical measurements. The question of how to integrate these types of measurement techniques into μCCA devices is being addressed [15,22,23].

Another challenge is that a common blood surrogate is required. Since different cell lines have different requirements for growth, this factor could become a limitation. In the devices developed so far, a common medium could be identified. This is, in part, due to the fact that cells can be initially cultured in their own medium on the chip prior to chip assembly and operation. Only when operating a chip is a common medium needed. Although it is challenging to find a common medium that is satisfactory for maintenance of the cell lines used, this factor has not been a significant limitation. Typically, the medium is supplemented with serum or a mixture of chemicals designed to mimic key functions of serum (e.g., a lipid mixture and serum albumin). This factor is necessary to emulate the transport properties of serum, especially for partially soluble substrates.

EXAMPLES OF A μCCA APPLIED TO CANCER

Cancer is a complex disease due to multiple mechanisms that play a role in its generation, growth, and spreading. It is not surprising that treatment with a single drug is often not successful. We are particularly interested in testing combinations of drugs that exploit different mechanisms and that might be more effective in combination than alone.

An animal study with a single drug with multiple dosing scenarios can be a complicated, expensive undertaking. Hence, testing potential combination treatments may become impractical with animals. Recognizing that it may be critically important whether drug A is given before B or vice versa and that the interval between drugs may be clinically important, a study that investigates a treatment that consists of four drugs each with five possible doses leads to a large experimental space. We believe a strategy that uses a PBPK-μCCA approach to explore a large experimental space can define the most

interesting candidate treatments and concentrations. Animal studies then could be carried out on this smaller subset of the experimental space.

To demonstrate the feasibility of testing combinations of drugs with μCCAs we have developed a μCCA aimed at treating MDR cancer [8]. Consider an example in which a patient may receive a chemotherapeutic and the tumor may even disappear. The cancer may reappear after a modest period of time (e.g., two years). The cancer that reappears is no longer responsive to either the original chemotherapeutic or a range of other chemotherapeutics. This MDR phenotype can occur due to multiple mechanisms, but the best-studied mechanism and possibly most common reason is overexpression of the membrane pump protein, P-glycoprotein. Cancer cells with MDR may have 50–100 times higher levels of P-glycoprotein than the original cancer cell line. This pump protein intercepts the drug before it can enter the cell and pumps it out. While MDR-suppressing drugs have been identified, none have passed clinical trials because of unacceptable side effects. However, it has been speculated that a combination of multiple MDR suppressors with one or more chemotherapeutics may be useful clinically by decreasing the severity of any single side effect [24,25].

To see whether we could create a μCCA that could be used to test such possible drug combinations, we created a μCCA with four cell-containing compartments [9]. One compartment contained a cell line (HepG2/C3A), representing the liver and metabolism of drugs. A megakaryoblast cell line (MEG-01) representing cells responsible for platelet formation was used in a "bone marrow" compartment. Another contained a "normal" tissue that might become dose-limiting. Two uterine cancer cell lines were used: MES-SA and its MDR variant MES-SA/DX-5. Using doxorubicin as the chemotherapeutic and two MDR suppressors, cyclosporine and nicardipine, the selective response in growth of the MDR cancer cells was monitored. Cyclosporine is used clinically as an immune system suppressor while nicardipine is a β-channel blocker. The MDR-suppressing activity of cyclosporine and nicardipine is incidental to their normal pharmacological use.

Using doses of doxorubicin that are only modestly higher than what is used clinically, the proliferation of each cell type in the μCCA was monitored. Using 72 h as the final time point, it was observed that the sensitive cancer cell line responded more strongly than the MDR cell line to 1 μM doxorubicin with more modest responses of the liver and megakaryoblast cell lines. When either cyclosporine (10 μM) or nicardipine (10 μM) were added in addition to doxorubicin (1 μM), the proliferation of the MDR cells was reduced from treatment with doxorubicin alone by 50 %, while the proliferation of other cell types was not altered significantly. More strikingly, when a combination of 5 μM nicardipine and 5 μM cyclosporine was used in place of 10 μM of either MDR modulator, we observed a further decrease in proliferation of the MDR cells to a net negative growth. This synergistic interaction of nicardipine and cyclosporine was not observed for any of the other cell lines. Nor was this synergistic interaction of these modulators observed when using multiwell plate assays. The interaction was only observed in experiments with the developed μCCA.

The observation of a selective synergistic interaction of MDR suppressors suggests that the use of a combination of MDR suppressors, as suggested earlier by Pascand et al. [24] and Lehnert et al. [25], might result in a useful therapeutic window. While we do not suggest that such a treatment be used, we believe it demonstrates that the μCCA technique could be used advantageously in a search for clinically used combination treatments.

As a second example of the use of the PBPK-μCCA approach to test a drug combination, we studied the case of colon cancer treatment with tegafur and uracil [9]. Tegafur is a prodrug of which the active drug, 5-flurouracil (5-FU), is released due to the enzymatic activity of various P450 monooxygenases located primarily in the liver. The compound 5-FU inhibits DNA and RNA synthesis in proliferating cells as well as inhibiting the enzyme, thymidylate synthatase. 5-FU is degraded fairly quickly in the body by the enzyme dihydropyrimidine dehydrogenase (DPD). Uracil is a competitive inhibitor of DPD and is added in combination with Tegafur to extend the length of time that 5-FU remains at an active level in the circulation. Because of differences in the level of DPD and other related enzymes in the human population, the therapeutically useful dose varies among individuals.

In the conducted experiment [9], we used a μCCA in which all cells are encapsulated in a hydrogel resulting in 3D constructs. The "liver" (HepG2/C3A) and "colon cancer" (HCT-116) cell lines were encapsulated in Matrigel^{TM}, and the myeloblast cell line (Kasumi-1) was encapsulated in alginate, which is a stiffer gel than Matrigel and which prevents cell migration out of the matrix. Using a 3D entrapment process increases the cell density, resulting in a more realistic liquid/cell ratio and encourages more authentic biological behavior. Results obtained with the μCCA were compared to those of traditional multiwell plate assays. While the multiwell plates could not predict the response to the prodrug, the μCCA responded analogously to the clinical observations. The response to tegufur plus uracil vs. tegufur alone in the μCCA was consistent with the role of uracil as a DPD inhibitor. The kinetics of the response to tegafur vs. 5-FU was similar to that observed clinically: the μCCA experiments predicted that hematological toxicity would be a more important side effect than hepatotoxicity. Thus, the μCCA demonstrated that a μCCA system with 3D constructs could be operated successfully.

DEVELOPMENT OF MICROFLUIDIC MODELS OF THE GASTROINTESTINAL TRACT

The two studies described above have focused on responses to the direct injection of the drug(s) into the systemic circulation. Often, drugs are not administered intravenously, but have to enter the body by crossing barrier tissues such as the skin, the lung epithelium, or the gastrointestinal (GI) tract. The way a drug is administered influences how much of it is absorbed, and its chemical design can be geared toward the planned uptake method. A drug's starting concentration needed to yield efficient concentrations at the target site can only be estimated with a μCCA if models of the barrier tissue in question are combined with systemic circulation models. Several barrier tissue models that are suitable for this purpose have been developed so far. Only some have been designed with physiological accuracy and used in combination with μCCAs. Among the candidate tissues are the blood–brain barrier, the lung epithelium, the microvascular endothelium, and the epithelium of the GI tract. Here we review efforts made toward developing and integrating analogs of the digestive system into the "body-on-a-chip" platform.

Oral intake is the most common way to administer drugs. It is easy to administer and because of that, patient comfort and compliance are usually high. Although the intestine with its enlarged surface is by nature "designed" for substance absorption, it presents physical and biochemical barriers to drugs present in the digest. Drugs designed for oral intake must, in addition to their therapeutic properties, possess properties that allow them to pass these barriers as well as the first-pass metabolism in the liver to reach systemic circulation. The degree to which the epithelium is permeable to molecules can be classified as leaky (gallbladder and small intestine), moderately leaky (colon and gastric antrum), and tight (gastric fundus and esophagus), depending on the resistance measured across the epithelial cell layer [26]. In addition to the barrier that is established by the presence of tight junctions between epithelial cells and the cell membranes themselves, drugs must cross the mucous layer consisting of heavily glycosylated proteins secreted by goblet cells that cover the apical membranes of epithelial cells [27]. Once a drug enters epithelial cells it comes in contact with oxidizing and conjugating enzymes that can alter it [28] and remove its therapeutic function [29]. Even if a drug is not rendered ineffective, drug exsorption can occur via transporter proteins present in the apical membranes of epithelial cells (P-glycoprotein). Upon passing through the epithelium, drugs cross the lamina propria and the endothelial cell layer lining the microvascular capillaries that deliver them directly to the liver, where they undergo a first cycle of metabolism that can greatly decrease the availability of the active compound.

In an effort to develop models that respond authentically, the cell culture component of the intestinal models developed in our group are based on co-cultures of multiple cell lines. Co-culturing Caco-2 cells and HT-29MTX cells at a ratio of 90–10 % establishes a mucous-covered epithelial layer in which both cell lines connect with each other to form tight junction complexes that give rise to the resistance across the cell layer characteristic of the human intestinal epithelium [30–32]. Caco-2 cells are enterocyte-like cells that develop microvilli that increase their apical membrane surface and facilitate

several molecular absorption routes: passive diffusion through the cells (transcellular), passive diffusion through the spaces between cells and the tight junction complexes located in those spaces (paracellular), energy-dependent carrier-mediated transport, and transport across the epithelium via vesicles (transcytosis) [33,34]. The mucus layer has been shown to present a significant barrier to the lipophilic drug testosterone [35] as well as to play a role in the uptake of metals such as iron [36,37]. The inclusion of goblet-like cells that produce mucous such as HT29-MTX therefore enhances the authenticity of the cell layer behavior. In addition, stimulation of 14–16-day-old Caco-2/HT29-MTX cell cultures with Raji B lymphocytes can initiate Caco-2 cell differentiation into microfold (M) cells that enable the additional route of drug transport via transcytosis that is also found in vivo. The Caco-2 cell model has been widely used in the pharmaceutical industry and in research to test drug absorption in static transwell systems. We used the co-culture system described above to study the absorption of iron from digestions of horse spleen, fish, and beef and have found that the model produces iron bioavailability data slightly lower than found in models that use Caco-2 cells only, indicating that the mucous layer decreases iron bioavailability [38]. The study suggests that including mucous-producing goblet cells may make the simulation more realistic.

Although the rate of movement of digest through the intestine can vary greatly and thereby affect the rate of absorption of drugs considerably, we think that microfluidic systems in which fluidic movement can be replicated, can simulate absorption conditions better than static systems in which no fluid movement takes place at all. It is well known that cells exposed to shear stress align themselves in the direction of flow and that a multitude of cellular processes from gene expression to signal transduction can be influenced by the presence of fluid flow. Our most recent study has shown that the presence of fluid flow can influence the location of digest components as indicated by 200-nm latex particles. Under static conditions they distribute evenly across the cell layer, but under fluidic conditions they accumulate at the cell–cell junctions. Overall, the results indicate that only about 8 % of 200-nm carboxylated latex particles cross the epithelial barrier and reach the basolateral side of the GI tract module. Most particles remain on the apical side of the epithelial cell layer outside the cells. This is likely due to the mucous layer secreted by the goblet cells. We think that the model is well suited to test drug absorption in general as well as the influence of nanoparticles on drug absorption and drug pharmacokinetics.

COMBINING μCCAS WITH MODELS OF THE GASTROINTESTINAL TRACT

The added effects of the physical barrier presented by the digestive system and the first-pass metabolism in endothelial and liver cells account for the largest concentration decrease of therapeutically active drugs that reach systemic circulation. It is of particular interest to simulate these processes together within a single model to capture synergistic effects resulting from metabolism in both organs. Models that simulate first-pass metabolism often combine Caco-2 cells with hepatic cells in transwells in which Caco-2 cells are cultured on porous membranes and HepG2/C3A cells are cultured in the chamber below. Experiments with such systems have shown that the two-organ response could be recreated in vitro. For example, while liver cells are sensitive to the chemical benzo[a]pyrene (B[a]P) and its metabolites, no significant reduction in liver cell viability was seen in the two-organ model, because Caco-2 cells transport the generated toxic metabolites back to the apical side [39]. Thus, the known low bioavailability of the B[a]P had been replicated in vitro. Another study by the same group has shown that the two-organ system can respond synergistically to a challenge with 3-methylcholanthrene (3-MC) [40]. The induced activity of the enzyme CYP1A1/2 was more elevated than would have been expected from the individual cell cultures. In an effort to reduce nutrient depletion over the course of the 48-h experiment, a simple fluidic circuit was constructed for this study. Another two-organ culture perfusion system that simulates first-pass metabolism has been developed by Brand and coauthors [41]. Using this system, the absorption of the peroxovanadium compound [VO(O2)2 1, 10 phenanthroline], short bpV(phen), and the subsequent increase of glucose consumption by Hep-G2 cells has been simulated. Remarkably, the authors also introduce a system in which a patch of mouse skin substitutes for the

Caco-2 cell layer to simulate the absorption of the compound through the skin. All these experiments are conducted with rather large amounts of media and drug concentrations per area covered with cells, and only some of the systems are capable of fluid recirculation. Often, the design/size of organ chambers is determined by the availability of resources to the researchers. Microfabrication has played a significant role in our ability to design and fabricate fluidic chambers of sizes that allow us to create chambers in which the ratios of cell types to each other as well as the ratio of fluid to cells is re-created physiologically correctly. Using this system, the digestion of acetaminophen has been simulated [42] and compared with existing in vivo data in mice [43]. We have found that both epithelial cells and liver cells metabolize acetaminophen, resulting in a dose-dependent decrease in liver cell viability. The results were within the range of those generated by a study of acetaminophen digestion in mice [43].

Although very useful, when pressure-driven flow is used to supply two-sided barrier tissues with media and drugs, the technical challenge of balancing fluid on either side of the circuit arises. Since tight junctions between cells could be interrupted, unnatural mechanical forces should not act on the model barrier tissue. Microfabrication techniques can be also used to include cell stress monitoring sensors into systems. Microelectrodes on each side of the cell layer, for example, could be used to measure the electrical resistance across the cell layer and indicate whether a barrier tissue has been damaged as a result of drug actions or as a result of fluidic imbalance (before drug administration). In general, the vast numbers of sensors developed for microfluidic systems will be beneficial to the development of μCCAs suitable for rapid drug screening. Prototype systems with incorporated sensors already exist (see Sin et al. [6] for a system with incorporated oxygen sensor).

CRITICAL LIMITATIONS OF μCCAS

There are critical limitations when using μCCAs for estimating the body's reactions to drugs. First, an in vitro system will probably never be able to capture the complexity of the human body in its entirety. While a μCCA with key organs is a realistic vision, representing all organs and barrier tissues of the human body on a single microfluidic platform is impossible when considering a realistic time frame. Second, at the cellular level, culturing immortalized cells as well as isolated primary cells in an environment outside the body can change their behavior. For example, cell layers consisting of Caco-2 cells only show good predictability for passively absorbed compounds, but poor correlation with in vivo data for carrier-mediated and paracellular transport of compounds [44]. An improvement can be achieved by co-culturing Caco-2 cells together with other cell types [45]. Further, the multicellular 3D architecture of organs is necessary for authentic cell function and must be replicated on chip if realistic simulation results with drugs are to be expected. For example, research in which growing tumor cells in 3D environments that provide inhibitory 1-integrin antibodies led to a striking morphological and functional reversion to a normal phenotype [46]. This reversion did not take place in 2D cell culture. These findings indicate that well-engineered 3D cell growth environments are necessary to achieve entirely authentic organ replicates. Many laboratories currently work on developing 3D tissue-engineered constructs that promise greater authenticity, and the inclusion of 3D constructs within μCCAs is possible [47]. Another possible solution to these problems might lie in the use of ex vivo tissue samples within μCCAs. Some results with precision-cut liver slices that were placed in perfused microfluidic systems have been achieved by van Midwoud et al., who tested the metabolism of 7-ethoxycoumarin and found that it correlates to data in 96-well plates as well as in vivo data [48]. Further research will need to be conducted to establish techniques that will allow the use tissue slices within μCCAs.

CONCLUSIONS

We have reviewed a class of in vitro cell culture models that provide useful tools for the screening of drugs and combinations of drugs in an inexpensive and rapid manner. The design of these models

(μCCAs) is based on mathematical PBPK models and attempts to represent the organs of the body in physiologically correct order and relationships to each other. The need to develop barrier tissues that can be integrated into such models is imminent, because drugs are often administered via oral, nasal, or other routes that present a barrier to the drug's absorption. Although several fluidic models of barrier tissues already exist, many lack authentic physiology such as the natural multicellular complexity of the tissue and often the physiologically correct drug residence time is not replicated. Among the barrier models developed so far, the digestive tract is one of the most common models, because the Caco-2 cell model is well established and is ready to be integrated into microfluidic systems. Models of the blood–brain barrier and the lung have been investigated, but have so far not been combined with other tissues. The development of models that simulate digestion and subsequent first-pass metabolism is particularly interesting, because these are two processes through which a drug may experience dramatic alterations, leading to a decrease in concentration of the active compound. Because each barrier organ adds an additional fluidic loop to the μCCA design, including several tissues with barrier function into μCCAs will make fluidic circuit design more challenging. Opportunities to improve μCCAs include improved in situ sensors for both physical and chemical measurements.

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