The Role of Body-on-a-Chip Devices in Drug and Toxicity Studies

M.B. Esch,[1] T.L. King,[2] and M.L. Shuler[1]

[1]Department of Biomedical Engineering, Cornell University, Ithaca, New York 14853; email: mls50@cornell.edu

[2]U.S. Army Engineer Research and Development Center, Construction Engineering Research Laboratory (ERDC-CERL), Champaign, Illinois 61826

Annu. Rev. Biomed. Eng. 2011. 13:55–72

First published online as a Review in Advance on April 20, 2011

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

This article's doi: 10.1146/annurev-bioeng-071910-124629

1523-9829/11/0815-0055$20.00

Keywords

microfluidics, in vitro tissue/organ mimics, live cell sensors, physiologically based pharmacokinetic models

Abstract

High-quality, in vitro screening tools are essential in identifying promising compounds during drug development. Tests with currently used cell-based assays provide an indication of a compound's potential therapeutic benefits to the target tissue, but not to the whole body. Data obtained with animal models often cannot be extrapolated to humans. Multicompartment microfluidic-based devices, particularly those that are physical representations of physiologically based pharmacokinetic (PBPK) models, may contribute to improving the drug development process. These scaled-down devices, termed micro cell culture analogs (μCCAs) or body-on-a-chip devices, can simulate multitissue interactions under near-physiological fluid flow conditions and with realistic tissue-to-tissue size ratios. Because the device can be used with both animal and human cells, it can facilitate cross-species extrapolation. Used in conjunction with PBPK models, the devices permit an estimation of effective concentrations that can be used for studies with animal models or predict the human response. The devices also provide a means for relatively high-throughput screening of drug combinations and, when utilized with a patient's tissue sample, an opportunity for individualized medicine. Here we review efforts made toward the development of microfabricated cell culture systems and give examples that demonstrate their potential use in drug development, such as identifying synergistic drug interactions as well as simulating multiorgan metabolic interactions. In addition to their use in drug development, the devices also can be used to estimate the toxicity of chemicals as occupational hazards and environmental contaminants.

Contents

1. INTRODUCTION

Developing new drugs is time consuming and expensive (1). The development time for a single drug often stretches over 12 to 15 years, and despite the generation of many potential drug candidates, only a few new drugs are approved for the market each year. It is estimated that only about 1 of 10,000 compounds tested is successful. Hence, over the past decade, the number of new approved molecular entities has lingered between 16 and 31 each year with a downward trend, and the number of approved biologic license applications has not exceeded 6 (2). These numbers represent only about 11% of compounds that promised potential in animal models and were allowed to enter clinical trials (3). Although efforts are made to estimate a drug's potential efficacy and toxicity with screening tools early in the development process, many drugs fail during full clinical development in Phases II and III (3), the most expensive phases of drug development. Unsuccessful drugs typically lack efficacy or are deemed not clinically safe because of toxic side effects (3). Especially high attrition rates occur in therapeutic areas in which traditional screening tools are not good representatives of human metabolism (4). Oncology drugs and drugs that treat diseases of the central nervous system are among the most affected candidates. Reducing drug development times and costs would be beneficial to pharmaceutical companies as well as patients and society as a whole. In the drug development process, high-throughput screening of compounds is an important step in identifying successful candidates. Both genomics and proteomics have aided in the identification of genes and proteins that are potential new drug targets. In addition, combinatorial chemistry has fueled the development of compounds that are potential drug candidates. The high number of synthesized compounds requires faster and more effective screening than can be done in animals to identify compounds that have the potential to succeed. Traditional screening tools, such as in vitro cell-based assays, give a first indication of the efficacy and toxicity of a compound. Compounds that were successful in in vitro tests can be evaluated further with animal models. In addition to these experimental tests, mathematical models that estimate a drug's distribution and its effects on the body guide drug development.

In the human body, a drug undergoes complex processes that involve its absorption, distribution, metabolism, and elimination (ADME) (5, 6). ADME processes determine the concentration-time profiles in the bloodstream and in the tissues of organs. Mathematical

pharmacokinetic (PK) models aim to estimate concentration-time profiles within each organ on the basis of the initial drug dose. Such mathematical models can be relatively simple, treating the body as a single compartment in which the drug distribution reaches a rapid equilibrium after administration. Two compartment models that distinguish between well-perfused organs such as the liver and kidney and poorly perfused organs such as muscle, skin, and fat are also widely used. Such models are mathematically simple and do not account for the underlying functional mechanisms that lead to ADME of a drug. More complete models, such as physiologically based pharmacokinetic (PBPK) models, assume separate compartments for each organ or tissue. Pharmacodynamic (PD) models are concerned with a drug's pharmacological effects as a function of administered dose. Models that combine PK or PBPK models with PD models can predict the time-dependent pharmacological effects of a drug. Mathematical models can be highly accurate when all parameters involved are known. However, compound-dependent variables such as tissue-to-blood partition coefficients and enzyme kinetic parameters can be difficult to estimate, especially for new compounds. In addition, data obtained from experiments with animal tissues do not reflect a drug's action accurately within healthy or diseased human tissues.

To evaluate a drug preclinically, researchers use well-established and well-characterized in vitro models as well as animal models. To test the efficacy and toxicity of cancer drugs, for example, the drugs are first tested for efficacy with numerous cancer cell lines (7) and for toxicity with cell lines derived from regular tissues. However, in vitro cell-based assays rely on immortalized cell lines that may no longer exhibit all functions the way they would in the human body. Cell culture plates or micro wells in which the cells are cultured do not provide a realistic model of the environment to which the cells are exposed in the body. The exchange of metabolites from one cell type to another, the three-dimensional extracellular matrix environment that provides chemical and mechanical cues, the physiological liquid-to-cell ratios in a tissue/organ, and the physiological shear stresses from fluid flow are all missing; therefore, authentic cellular behavior is limited. The assays also do not consider the complexity of the body with its multiple organs and their interactions, which alter ADME.

If the drug is evaluated positively with cell-based assays, more advanced tests with animal models are conducted. As an example, for a potential oncology drug, the hollow fiber assay is used to test the drug's metabolic activity. In this assay, cancer cells are placed into semipermeable fibers and introduced into mice intraperitoneally or subcutaneously (8). Drugs that prove effective in reducing tumor cell growth within these mice are then tested further with mouse xenograft models. In these models, human tumor tissues are implanted directly into mice, and the efficacy of the drug is determined (9). These tests provide some indication about a drug's efficacy. However, a comparison of estimated and actual efficacy of drugs in humans shows that animal models do not always predict the actions of drugs accurately (10). This arises from the facts that human metabolism and animal metabolism can differ considerably, that the tumor is implanted rather than spontaneous, and that immunocompromised animals are typically used. Even extensive preclinical testing with in vitro assays and animal models does not guarantee clinical efficacy and the absence of toxicity. Indeed, because oncology drugs have a success rate of only 5%, it is clear that animal models are only marginally effective. As a result, using an extensive number of animals to study the actions of drugs also gives rise to ethical concerns, especially because animal results are not that predictive.

In vitro models and animal models that simulate the human metabolism accurately could help in increasing the number of drugs for which predictions are in accordance with their effects in clinical trials. Because animal metabolism is different from human metabolism, it is possible that drug candidates are eliminated even though they might be effective in humans. Better in vitro models of human and animal metabolisms would be useful in selecting drugs to be tested as well as

identifying the appropriate concentration ranges. Such models would increase the predictability of human response and lead to higher success rates in clinical trials. This factor would reduce expenses and speed up the drug's development. More accurate estimations of the efficacy and toxicity of a drug would help in identifying successful drug candidates early in the development process and in eliminating those that will not lead to a successful product. Developing assays that build on the advantages of in vitro screening tools and refining them would increase their predictive power. Furthermore, more mechanistically based information that could be included in mathematical models could potentially increase their predictive power and thereby reduce the parameter space in terms of drug concentrations and combinations that need to be tested with animal models and in clinical studies. Ideally, a more realistic in vitro model that mimics animal and human response accurately would reduce dependency on animal studies and identify drugs that are more likely to be successful in clinical trials.

2. APPROACHES TO IN VITRO MULTICOMPARTMENTAL/ MULTIINTERACTING TISSUE SYSTEMS BASED ON MICROFLUIDIC PLATFORMS

One of the limitations of conventional single-cell-type-based assays is that they do not capture multiorgan interactions and realistic dose dynamics. Metabolites generated in the liver, for example, can influence the function and gene expression in other cell types and vice versa. This has been seen in coculture assays in which several cell types are cultured within the same culture chamber (11–13). Efforts have been made to develop systems in which several tissues (represented by cell lines) can interact with one another via metabolites that travel between cell culture chambers via medium flow. We have reviewed recently microfabricated devices that have been used with multiple cell types. Here we give selected examples, but a more detailed discussion can be found in Sung & Shuler (14). Cross-talk between tissues is realized through mechanisms that occur in the human body, i.e., through soluble agents. Li et al. (15) developed a multiwell system termed well within a well. In it, various cell types are cultured isolated from one another but are connected via a common medium. In demonstrations of its feasibility, this system has been utilized to evaluate the toxicity of tamoxifen (an anticancer drug) toward primary hepatocytes, kidney cortical cells, small airway epithelial cells, astrocytes, aortic endothelial cells, and MCF-7 human breast adenocarcinoma cells. Another device that demonstrated metabolism-induced cytotoxicity is that developed by Ma et al. (16). This device is a microfluidic device that contains two separate chamber arrays. Sol-gel-encapsulated human liver microsomes in the first chamber facilitated the metabolic conversion of acetaminophen, and drug interactions between acetaminophen and phenytoin and their influence on HepG2/C3A liver cell viability were recorded in the second chamber array. The systems by Ma et al. (16) and Li et al. (15) are capable of re-creating cell-cell communication, although the rate of exchange of metabolites is not physiologically realistic. Building on this idea, Vozzi et al. (17) developed a system that demonstrates cell-cell interactions with consideration of physiologically correct representation. Murine hepatocytes and human umbilical vein endothelial cells were cultured within two separate chambers on a microfluidic platform. Increases in endothelial cell viability, hepatic glucose synthesis, and albumin and urea production as well as downregulated lactate production could be attributed to the exchange of solutes between the cell culture chambers. Thus results that have been obtained with simple coculture systems could be re-created in a multichamber fluidic device, demonstrating the concept of a multiorgan micro cell culture system.

Zhang et al. (18) have focused on creating a cell culture environment that is capable of multicompartment interaction but that limits the interaction to diffusion. The multiorgan chamber device contains side channels that supply medium to the liver, lung, kidney, and adipose chambers.

Mass transfer between medium and cells in this device is more limited than in devices in which the cell culture chambers are directly perfused with medium. Although it limits the exchange of metabolites, this approach could potentially facilitate longer operation times because toxic waste products from one compartment travel to other tissue compartments in lower concentrations.

The use of microfluidic fabrication technologies has been critical in the creation of micro cell culture analog (μCCA) devices that aim to create models that replicate aspects of the human body as closely as possible. Although creating a correct model of the entire body is an immense challenge and has not been realized in any single device, using microfluidics has brought us closer to this goal than has any other technology. Originally developed for the semiconductor industry, microfabrication techniques are being adapted to create devices with structures of sizes that are relevant to biological systems such as cell sizes (on the order of 10 μm) and the sizes of human blood vessels with spacing between capillaries on the order of 100 to 200 μm. At these sizes, we are able to create more physiological growth environments with relevant shear stress, liquid-to-cell ratios, and physiological fluid residence times in tissue compartments. Several studies have shown that the application of shear stress influences cellular function and gene expression (19, 20), and this parameter can be designed into such devices. Microfabricating devices also allows us to custom-design them and scale the organs' compartments correctly with respect to one another. Fluidic conduits represent blood flow, which can be recirculated to allow for the exchange of metabolites in a way that mimics physiological conditions. Only small quantities of reagents and cells are needed, and parallel processing makes relatively high-throughput screening possible.

In the development of multicompartment devices, representations of the human body such as those in used PBPK models can be used to guide the device design with regard to the arrangement of chambers and fluidic channel connections. When PBPK models are used as guidance in the design, the resulting systems are physical representations of PBPK models. The cells represent organs and conduct the reactions described in the model with equations, and fluidic flow represents transport via the bloodstream. In this way, the metabolic and biochemical characteristics of the human or animal body can be achieved. Devices that are designed with regard to the structure of the human body are known as μCCAs or body-on-a-chip devices (21, 22).

PBPK models and μCCAs can be used in conjunction to inform each other. Because PBPK models rely on the input of already known mechanisms, a complete model would include all reactions and pathways involved in a drug's metabolism. However, we rarely know all reactions. Additionally, reactions within diseased tissue may differ considerably from those in healthy tissue. In a μCCA, the different cell types represent what is described in the model by mathematical equations. Thus the physical representation is a more complete model because the cells may exhibit relevant reactions that may not be included in the PBPK model. The μCCA concept combines attributes of a PBPK model and other in vitro systems. Data obtained with these systems may be used to test and refine mechanistic hypotheses. Conversely, the mathematical model also can be used to analyze and interpret data obtained with μCCAs. The PBPK model can be made an exact replica of the μCCA; the predicted response and measured μCCA response should exactly match if the PBPK model contains a complete and accurate description of the molecular mechanisms. In the μCCA, all flow rates, the number of cells in each compartment, and the levels of each enzyme can be measured independently, so that no adjustable parameters are required. If the PBPK predictions and μCCA results disagree, then the description of the molecular mechanisms is incomplete. The μCCA and PBPK model can be used in an iterative manner to test modifications in the proposed mechanism. When the PBPK model is extended to describe the whole animal, failure to predict animal response would be due to inaccurate description of transport (particularly within an organ), inability to accurately measure kinetic parameters (e.g., in vivo enzyme levels or activities), or the presence of in vivo or metabolic activities that are not present in the cultured

cells or tissues. Advances in tissue engineering will provide tissue constructs to use in a μCCA that will display more authentic metabolism than that displayed by isolated cell cultures. In addition, unlike other in vitro systems, the μCCA is an integrated system that can mimic dose dynamics. μCCAs can be used to predict human pharmacological response to drugs and assess risk due to chemical exposure. If an animal μCCA could make accurate predictions of animal experiments and if the results were in accordance with PBPK predictions, we would have an indication that the concept of μCCAs is valid. If we use the same approach to constructing a human PBPK model and μCCA for the same compound, then we would have a rational basis to extrapolate animal response to predict human response when human experiments would be inappropriate. Also, because the PBPK model is mechanistically based, it would provide a basis for extrapolation to low doses. The μCCA/PBPK approach complements animal studies by potentially providing an improved basis for extrapolation to humans (see **Figure 1**).

Furthermore, data from μCCAs validated as described above could provide parameters for PBPK models for single compounds. If a PBPK model for compound A and a PBPK model for compound B are combined, then the response to any mixture of A and B should be predictable because the mechanisms for response to both A and B are validated with μCCAs and included in the model. Predictions of human response to mixtures of drugs or chemicals whose interactions may be synergistic or antagonistic would thus be based on experimentally validated models. The predicted concentrations of interest could then be tested experimentally.

3. MONITORING THE RESPONSE OF CELLS WITHIN MICROFLUIDIC SYSTEMS TO CHALLENGES WITH DRUGS OR ENVIRONMENTAL CHEMICALS

Microfluidic cell and tissue culture systems present analytical challenges owing to their small size, low cell numbers, and physical inaccessibility. In principle, any detection technology can be chosen for the analyte or process of interest as long as the technique can be executed within the engineering constraints of the cell culture device. Unlike larger connected culture or coculture systems in which sample aliquots can be withdrawn to measure analytes such as glucose, albumin, or urea (17), the small volumes and inaccessibility of the recirculating fluid on μCCA chips limit the utility of traditional analysis methods that rely on removing significant amounts of sample from the system. The small physical dimensions of microfluidic culture systems inherently contain small numbers of cells, leading to mass-limited quantities of analyte molecules and placing further constraints on the required sensitivity of analytical methodologies. Optical and electrical techniques have found extensive application in these devices owing to their ready compatibility with microfluidic formats and relative ease of operation. Such systems can provide near-real-time analysis. Microfluidic systems in general and μCCAs in particular are ideally suited to optical interrogation owing to the noninvasive nature of optical measurements. The optical transparency of many common device materials such as polydimethylsiloxane, poly(methyl methacrylate), and glass readily accommodates optical measurements, and the sizes of both microfluidic device features and eukaryotic cells are commensurate with the size scales accessible via optical microscopy. Many electrical measurement methods such as amperometry with enzymatic bioelectrodes and cellular impedance are compatible with μCCA devices owing to the ease with which electrodes can be integrated into device structures.

Many experiments carried out on μCCAs simply aim to know whether the compound of interest affects the survival of cells. For example, a μCCA containing amphibian tissues could be used by environmental regulators to rapidly screen environmental water samples of unknown composition for deleterious effects on wildlife. Cell viability has been optically measured on μCCA devices by

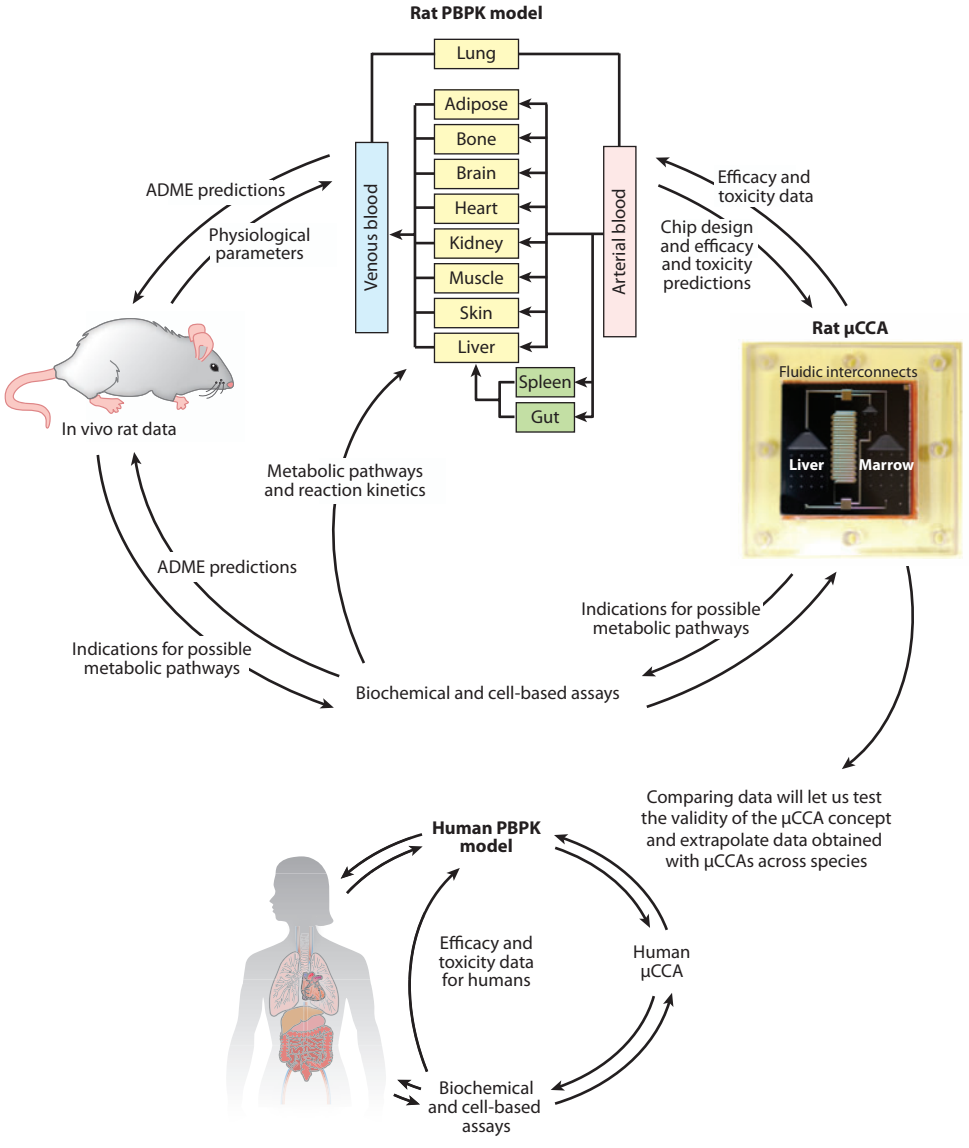

Figure 1

Schematic diagram of how microfluidic cell culture systems can be used in conjunction with other in vitro cell-based assays, mathematical models, and in vivo experiments to enhance the drug development process and improve toxicity estimations for environmental contaminants. For example, comparing data obtained from a micro cell culture analog (μCCA) operated with rat cell lines with data obtained from a rat physiologically based pharmacokinetic (PBPK) model and in vivo rat data will let us test the validity of the μCCA results. Our understanding of how these results can be used in conjunction with PBPK models and in vivo experiments will let us draw conclusions about how the devices could be used to better predict efficacy and toxicity of drugs in humans. Abbreviation: ADME, absorption, distribution, metabolism, and elimination.

observing exclusion of trypan blue (21), uptake of CellTrace blue (23), and live/dead staining with a combination of calcein AM (live) and ethidium homodimer-1 (dead) (24). The primary disadvantage of experiments relying solely on indicators of cell viability is that little insight into the biochemical mechanisms of any observed toxic response is available. More specific information can be obtained either by utilizing unique spectral signatures of compounds of interest or by introducing selective reporter systems. The integration of ultraviolet-visible absorption spectroscopy with microfluidic cell culture chips has been used to study acetaminophen metabolism and cytotoxicity (16). Specific knowledge of metabolic function also can be obtained by leveraging the inherent selectivity and specificity of fluorescence-based assays through the use of fluorescent or fluorigenic reporter molecules. For instance, the fluorigenic reagent monochlorobimane has been used to measure reductions in intracellular glutathione due to toxicant-induced oxidative stress (25, 26). However, as monochlorobimane binds to glutathione, only end-point assays can be conducted, requiring multiple experimental runs to acquire a time series. Autofluorescent substrates, although not available for many experimental systems, are ideally suited to μCCA-based assays owing to the simplicity of their analysis. Fluorescence microscopy has been used to show cellular uptake of the autofluorescent chemotherapy drug doxorubicin (23) and localized bioaccumulation in adipocytes of the autofluorescent polycyclic aromatic hydrocarbon fluoranthene (25). Highly specific reporters of cellular response can be engineered through the creation of reporter cell lines. For example, multiple cell lines containing an estrogen-sensitive green fluorescent protein (GFP) reporter system were incorporated within the organ compartments of a μCCA to study the effects of endocrine-disrupting chemicals on estrogen production (27).

The simplicity of including microfabricated electrodes on a μCCA chip has the potential to facilitate a range of electrical measurements from highly specific metabolic assays, such as amperometry with enzymatic bioelectrodes, to more general assays, such as cellular impedance measurements, to determine viability. Although neither of these techniques has been demonstrated within a μCCA, both have been integrated into other microfluidic cell or tissue culture systems. Microfabricated bioelectrodes could be integrated into μCCA substrates easily and provide sensitive, continuous, label-free measurement of many analytes of interest including superoxide (a marker of acute oxidative stress) (28), lactate (a marker of anaerobic respiration) (29), and others. Electrical impedance measurements are a particularly attractive option for determining cell viability in microfluidic cell culture devices because they are real time, label free, and noninvasive. In impedance-based assays, cells are seeded atop electrodes or electrode arrays. After cell adhesion and proliferation, electrochemical impedance spectra of the entire system are recorded. Changes in impedance are then correlated to changes in the number of cells atop the electrodes (30). Like live/dead staining, typical impedance measurements give a measure of cell viability without providing biochemical information on the causes of cell death. However, some reports suggest that detailed biochemical information can be ascertained from impedance data (30). Impedance measurements have been used in conjunction with cell or tissue culture chips for the study of individual toxicant response (31, 32), drinking-water toxicity (33), nanoparticle toxicity (34), and other causes of cell death (35).

Multiplexing μCCA-based experiments is an important goal in realizing the full potential of this experimental platform. Many pharmaceutical and toxicology studies must cover large parameter spaces, potentially requiring many μCCA chips to be run in tandem. Although hardware to multiplex electrical measurements is readily available and relatively simple to engineer, multiplexed optical assays require specialized hardware development. Toward this end, a modular fluorescence microscope has been created to allow in situ fluorescence imaging of μCCA chips within the confines of an incubator (36). Another version of this system was used to image multiple μCCA chips for more than 90 h to observe long-term cell proliferation (37).

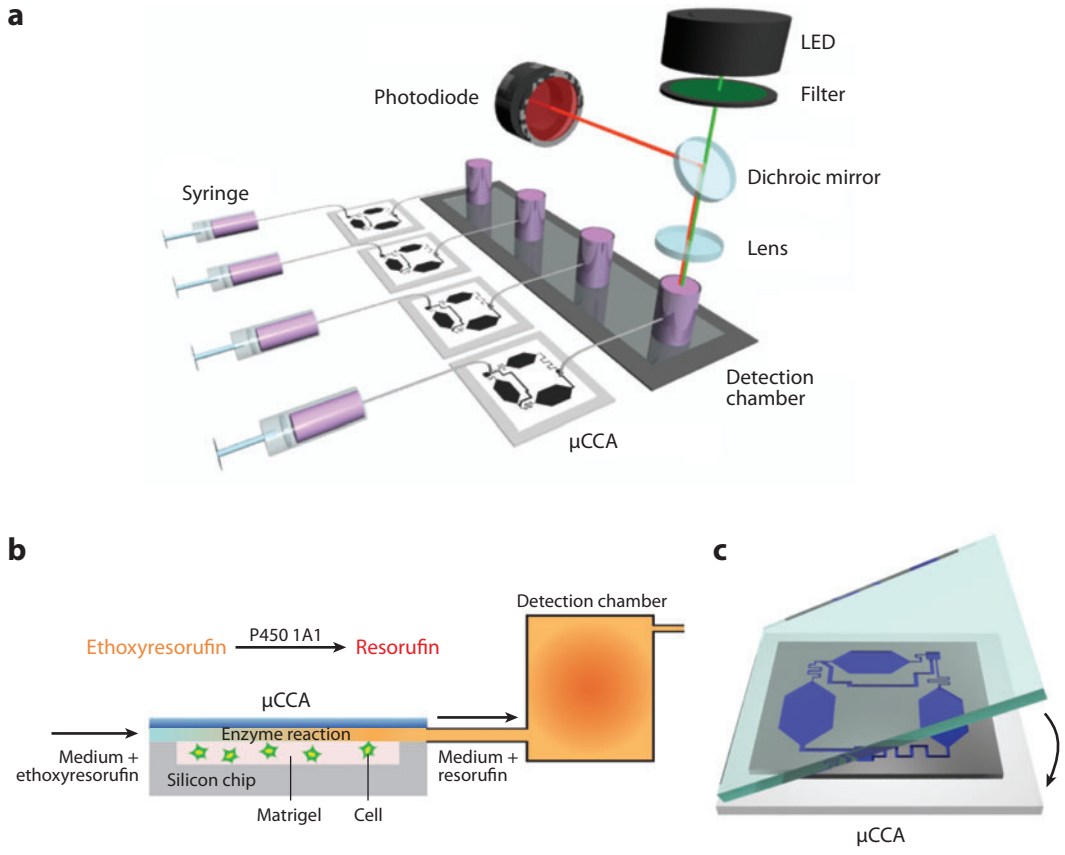

Figure 2

(*a*) In situ fluorescence optical detection system (ISFODS). A schematic diagram of the whole system is shown, consisting of a syringe, micro cell culture analog (μCCA) chips, and the ISFODS. The ISFODS consists of a photodiode, a light-emitting diode (LED), a filter, a dichroic mirror, a lens, and detection chambers. The whole system is placed inside an incubator. Medium-containing substrate is perfused into a μCCA, and the fluorescent product is detected at the detection chamber located below the lens. (*b*) A cross-sectional view of a μCCA, with cell-embedded Matrigel™. During the experiment, medium-containing substrate (ethoxyresorufin) is perfused above the hydrogel matrix of liver cells. The substrate diffuses into the hydrogel, and P450 enzymes present in the liver cells convert the substrate into a fluorescent product, resorufin, which is released into the flow medium. The medium flows through a detection chamber, where the detection of fluorescent signal is made. (*c*) A diagram of a μCCA device assembled with top and bottom frames. A μCCA consists of a liver, tumor, and marrow chamber (38). In this study, only the liver chamber was used; the other two chambers were left empty. A polycarbonate top frame is closed and secured with screws for complete sealing. Reproduced with permission from John Wiley and Sons.

In a more sophisticated implementation, an optics and detection module mounted to a translation stage is moved between multiple μCCA chips within an incubator to create an in situ fluorescence optical detection system (ISFODS), providing functionality similar to that of a conventional fluorescence microplate reader (38). This system was used to monitor the conversion of ethoxyresorufin (nonfluorescent) to resorufin by P450 enzymes within liver cells on the μCCA chip (**Figure 2**).

Development of detection methodologies and instrumentation applicable to these analytically challenging systems lags behind that of other design aspects such as three-dimensional tissue culture, fluidic control, and reagent delivery. As a result, the broad range of chemical information

contained within microfluidic culture chips is not fully accessed by currently employed techniques. Further development in this area could provide a wealth of novel information regarding the response of complex, multicomponent biological systems to toxic environmental assault or novel pharmaceutical therapies.

4. EXAMPLES OF APPLYING IN VITRO MICROFLUIDIC MIMICS TO DRUG TESTING AND TESTING CHEMICALS FOR TOXICITY

One of the main advantages of μCCAs is their ability to simulate the exchange of metabolites between organs. This ability has been demonstrated with several μCCAs; here we focus on a system that contained liver cells (HepG2/C3A), colon cancer cells (HCT-116), and myeloblasts (Kasumi-1) (24). The device was operated with and without Tegafur, an oral prodrug of the cancer drug 5-fluorouracil (5-FU). Administering Tegafur instead of 5-FU to patients provides a more stable concentration of the effective component 5-FU because Tegafur's bioavailability is better than that of 5-FU alone. Upon oral uptake, the enzyme dihydropyrimidine dehydrogenase rapidly degrades 5-FU, resulting in a low response rate (39). The μCCA was able to reproduce the metabolism of Tegafur, i.e., its conversion to 5-FU in the liver cell compartment. 5-FU traveled through the microfluidic connections to the cancer cell compartment and caused a decrease in cell viability. This result was not seen in 96-well plates using the HCT-116 cells dosed with Tegafur because the prodrug was not converted into active 5-FU.

μCCAs are relatively inexpensive devices and can be used to test a large number of drugs as well as their combinations at varying concentrations. A combination of drugs might be beneficial when one drug alone does not treat a disease effectively or when this drug would have to be administered at doses that would cause unacceptable side effects. Drugs that have similar functions but different side effects potentially could be combined at lower dosages so that treatment is still possible. Using such drug combinations might prove particularly important for the treatment of diseases such as multidrug resistant (MDR) cancer. One mode of multidrug resistance is when cancer cells intercept normally toxic drugs so they cannot enter the cytoplasm at an effective concentration. Chemical modulators can inhibit the actions of the membrane transporter P-glycoprotein that facilitates this transport (40). However, when administered as single drugs, the high modulator concentration caused toxic side effects in clinical studies. It has been hypothesized that combinations of lower doses of multiple modulators can be combined effectively with chemotherapeutics to reverse the growth of MDR tumors without unacceptable side effects (41, 42). Using a μCCA, Tatosian & Shuler (23) tested combinations of drugs on two cancer cell lines (uterine cancer MES-SA, and an MDR variant of uterine cancer MES-SA/DX-5 that overexpresses P-glycoprotein). The device also contained megakaryoblast cells that form platelets (MEG-01) and liver cells (HepG2/C3A). Experiments showed that combining the chemotherapeutic doxorubicin with the MDR modulators cyclosporine and nicardipine is more effective in inhibiting cancer cell proliferation than using doxorubicin alone or with only one of the two modulators where the total dose of all modulators was kept constant. Liver cells and bone marrow cells (i.e., MEG-01) were not affected by the drug combination compared with the use of a single MDR modulator. This result was contrasted with those obtained in 96-well plates that suggested an additive effect rather than a synergistic effect as seen with the μCCA. As with the study of multidrug combinations for cancer treatment, one can imagine that individualized drug treatment could be devised for patients using biopsy cells.

Similar to drug toxicity testing, testing of chemical toxicity can be time consuming and expensive. μCCAs can facilitate the estimation of hazards due to environmental contaminants. A proof-of-concept device was designed on the basis of a rat PBPK model and used to probe for the toxicity of naphthalene (26). A four-chamber, two-cell-type system was developed for this purpose.

Naphthalene, the primary ingredient in mothballs and a precursor for the synthesis of other chemicals, was chosen as an example of a polycyclic aromatic hydrocarbon compound. These compounds are environmental contaminants that occur in coal tar and are by-products of fuel burning. When naphthalene is taken up into the human body, cytochrome P450 enzymes in the liver convert it into naphthalene epoxide. Through several pathways, naphthalene epoxide reacts further into metabolites (1,2-naphthalenediol and 1,2-naphthoquinone) and reactive oxygen species that can cause oxidative stress in tissues (43). The produced metabolites conjugate to glutathione, thereby depleting glutathione levels. Glutathione is an antioxidant that, when present, prevents damage to cellular components. The μCCA device, operated with two cell types representing the liver (H4IIE rat hepatocytes) and lung (L2 rat lung type II epithelial cells), showed that the addition of naphthalene to the medium in the μCCA causes glutathione level depletion, resulting in lung cell death. These results are in accordance with observations in mice (44) as well as with corresponding PBPK models (45). Because the half-life of naphthalene epoxide in aqueous medium is 3.6 min (46), and the residence time in the other tissue/debubbler compartment is approximately 50 min, the results obtained with the μCCA support suggestions that naphthoquinone causes the observed toxicity (47, 48) rather than naphthalene epoxide, as was assumed previously (49–52). A later addition of differentiated 3T3-L1 adipocytes to the μCCA suggested that storage of naphthalene and naphthoquinone in fat tissue reduces glutathione depletion in the lung compartment, reducing the toxic effect (25).

Another study with environmental toxicants was conducted by Xu et al. (27). Here, a system with two cell lines (MCF-7 human breast cancer cells and endometrial carcinoma Ishikawa cells) was cultured within a μCCA that was probed for a response to estrogenic endocrine disruptors. Estrogenic pollutants are a growing health concern because they can mimic natural hormone estrogens and cause developmental and reproductive diseases as well as increase the incidence of estrogen-related cancers (53–55). They occur in pesticides (56, 57), plasticizers (58, 59), and food antioxidants (60), as well as some herbs. Estrogen has different effects on different organs. It binds to specific sequences in the genome either through liganded estrogen receptors or through interactions with other DNA-bound transcription factors such as activating protein 1 (61–63). An interesting approach to measuring the response of cells to the toxicants via GFP production was provided. Both cell lines were infected with a two-tandem estrogen response element–E4 promoter-GFP reporter gene construct. Tests with a variety of estrogenic compounds (17β-estradiol, diethylstilbestrol, and 2-hydroxyestradiol) and environmental toxins (bisphenol A, genestin, and o-p′-DDT) showed that both mammary and endometrial cancer cells responded in a dose-dependent manner. Compound binding caused GFP production in both cell lines in response. Raloxifen and tamoxifen acted as active antiestrogens in MCF-7-GFP cells but as partial estrogen receptor agonists in Ishikawa-GFP cells. The cells were also tested for synergistic responses, which the study concluded were not present.

5. THE IMPORTANCE AND CHALLENGES OF INCORPORATING BARRIER TISSUES WITH SYSTEMIC CIRCULATION

The μCCAs discussed so far simulate the intravenous administration of drugs and toxicants. However, barrier tissues such as the epithelium of the gastrointestinal (GI) tract, the lung epithelium, the skin, and the blood-brain barrier can significantly reduce the bioavailability of drugs that are taken up orally, through inhalation, or through application to the skin. Oral delivery is the preferred route of delivery because administration is easy and patient compliance and comfort are high. However, the mucous-covered epithelium, as well as the lamina propria and the endothelial lining of the blood capillaries, significantly reduces the concentration of drugs that

arrive at the target site (64, 65). After traveling through the GI tract, compounds immediately reach the liver, where metabolic reactions take place before further distribution in the body (66). This process is termed first-pass metabolism and has been re-created in vitro with static and fluidic models. In static models, Caco-2 cells grow on a transwells membrane, and hepatocytes grow in the well beneath the membrane (67, 68). A common medium supply that covers both wells connects both cell cultures so that metabolites generated in the Caco-2 cell culture can diffuse to the liver cell culture and vice versa. These models rely on relatively large liquid-to-cell ratios and do not provide recirculation of medium. In a model in which fluid flow is present, metabolites are transported from the GI-tract cells to the liver cells located downstream (69). Two models that recirculated fluid were developed by Choi et al. (70) and Mahler et al. (71). Mahler et al. also included HT-29 goblet-like cells in the GI-tract compartment so that the Caco-2 cell layer was covered with mucous (72). The model responded to a challenge involving acetaminophen with liver cell toxicity in a dose-dependent manner, and acetaminophen metabolites were detected in the medium. Acetaminophen is a widely used analgesic and antipyretic drug that can cause hepatotoxicity when taken at an overdose. It is a small, nonionized molecule that passively diffuses across the GI-tract epithelium. The presence of the GI-tract barrier in the μCCA model reduced liver cell death because acetaminophen diffused slowly across the mucous-covered Caco-2/HT-29 cell layer, and, in addition, intestinal cells also converted some of the drug into nontoxic metabolites (71). The result obtained with the first-pass metabolism model was consistent with those obtained with acetaminophen challenges in mice (73).

Occupational and environmental hazards often are related to the uptake of toxic substances through the lung. A model of the gas-exchange surface of the lung that correctly captures the tissue oxygenation and carbon dioxide clearance could be used to simulate the uptake of drugs or toxins through the lung epithelium. However, engineering the alveolar-pulmonary barrier of the lung is challenging. The barrier consists of alveolar air spaces with walls that are lined with epithelial cells. A basement membrane consisting of extracellular matrix proteins such as type IV collagen, laminin, and a mix of glycosaminoglycans separates the epithelium from the underlying endothelium of the lung microvasculature (74, 75). The ratio of cell volume to extracellular fluid volume is exceptionally small compared with other tissues. Respiration exerts great mechanical stresses on the lung epithelium because the airflow deforms the alveolar-capillary interface. Modeling the lung epithelium (A549 cells) and endothelium (human microvascular endothelial cells, or HMEC-1 cells) within microfluidic channels showed that both cell types were sensitive to shear stress (76). Bilek et al. (77) have grown lung epithelial cells within parallel plate devices and were able to simulate lung cell damage due to surface tension during air-bubble travel. Huh et al. (78) were able to re-create the air-liquid interface and grow cells within this environment. The crackling sound that is a symptom of pulmonary disease, often caused by the lack of pulmonary surfactant in lungs affected by disease, could be re-created on a chip. This model was followed by a model that includes mechanical forces (79). Mechanical forces are important for many cell types, and the lung tissues are stressed mechanically to a greater extent than most tissues (80).

Among the barrier tissues, the blood-brain barrier is particularly difficult to re-create in vitro. Primary or low-passage-number brain endothelial cells are best suited to re-create this particularly restrictive barrier. The tight junctions between the cerebromicrovascular endothelial cells allow for oxygen and carbon dioxide diffusion (81) but restrict intercellular permeability so that most blood-borne substances do not pass the barrier. The transendothelial resistance has been measured to be as high as 1,000 Ω cm^2 (82–84). In static models of the blood-brain barrier, brain endothelial cells and astrocytes are cocultured in transmembrane wells (85). The coculture of astrocytes and endothelial cells is thought to provide important cell-cell stimuli for the establishment of the barrier function (23, 86, 87). Commercially available

membranes, however, do not allow for a close interaction of astrocytes and endothelial cells because their porosity is low. Microfabricated membranes such as that developed by Ma et al. (88) allow for better interaction between the cells as well as the application of fluidic flow, which has been hypothesized to play a role in the functional differentiation in brain endothelial cells (89). On-chip fabricated membranes also enable the integration of models of the in vitro blood-brain barrier with other organ analogs to construct a μCCA that can be used to test drugs that target diseases of the nervous system.

6. INCREASING THE POTENTIAL IMPACT OF MICROFLUIDIC IN VITRO SYSTEMS

To increase the impact and acceptance of μCCAs, several factors need to be addressed. First, devices must achieve authentic cellular behavior (i.e., cellular behavior that is identical to that seen in vivo) and produce realistic results to be most useful to drug developers and clinicians. Cell lines do not always satisfy this requirement. For example, cells from immortalized liver cell lines (HepG2) contain lower concentrations of metabolizing enzymes than do primary human hepatocytes (90). The use of primary cells or tissue slices could address this problem, and the use of primary human hepatocytes has been demonstrated on body-on-a-chip devices (94). More authentic cellular behavior also is facilitated by methods that can be used to make three-dimensional tissue constructs such as simple hydrogel encapsulation of cells or mixtures of cell types. More realistic systems using preformed scaffolds are also possible and may represent a high level of authenticity, particularly for autocrine-like compounds. Although most work with μCCAs has utilized two-dimensional cell cultures, a few devices have been operated with gel-encapsulated cells (24). Methods that enable the storage and revitalization of cells and tissue slices would enable the routine use of μCCAs in clinical laboratories and for on-site testing for environmental contamination. Also, each cell line has an optimum cell culture medium, and developing a common medium that can act as a blood surrogate in all tissue compartments is challenging. A solution to this problem has been proposed by Zhang et al. (18), who supported hepatocyte cell growth in a multiorgan device with controlled-release, gelatin-encapsulated transforming growth factor beta 1 (TGF-β1). TGF-β1, released from fibroblasts, has been shown to enhance hepatocyte function in cocultures (91). When released from gelatin microspheres within a hepatocyte cell culture chamber on a microfluidic device, TGF-β1 enhanced the sensitivity of the cells toward acetaminophen (92).

Another advance that is needed is to make systems that are easy to operate. Most prototype devices reviewed here were operated with relatively large equipment such as incubators, syringe pumps, or peristaltic pumps. Peristaltic pumps often are operated with flexible polymer tubing that can absorb compounds (especially hydrophobic ones), leading to significant chemical loss when the fluid is recirculated (95). Possible solutions to this problem include the development of nonabsorbent tubing (96), on-chip micropumps (97), or pumpless systems such as that developed by Sung et al. (93). The last two approaches also simplify the operation of microfluidic devices while still providing flow rates that create hydrodynamic shear stress in the physiological range as well as providing physiologically relevant values of residence times in the organ compartments. They are also a step toward the portability of the devices and enhance the potential for increased throughput and multiplexing.

Perhaps the most important factor in achieving more widespread acceptance is to test the validity of results obtained with μCCAs. This process entails building an animal μCCA and comparing the results obtained via that device with those obtained via animal studies and animal cell line data (from the same animal). Human μCCAs (operated with human cell lines) also can

be used to test drugs for which clinical efficacy and toxicity data exist. Both methods will help us in developing an understanding of the similarities and differences between each method and how they could be used in conjunction with each other. This knowledge will then aid in interpreting and extrapolating data obtained with μCCAs across species.

7. CONCLUSIONS

Mathematical simulations and in vitro screening devices are useful tools in drug discovery and development. To improve the predictive power of in vitro screening tools, physical representations of mathematical models termed μCCAs or body-on-a-chip devices have been developed. Experiments with several prototype systems have shown the feasibility of these systems to study the efficacy and toxicity of drugs and environmental toxicants. The actions of metabolized drugs as well as synergistic drug actions have been mimicked successfully in μCCAs. We envision that microfluidic cell culture systems such as μCCAs could be used in conjunction with PBPK models to augment the drug development process, resulting in increased success in clinical trials. To make the devices more useful, barrier tissue analogs need to be developed and included. More authentic cell culture environments that lead to more authentic cellular behavior would also be desirable. Microfluidic devices are well suited for this purpose, because their natural length scale and that of mammalian systems (ca. 10–100 μm) are the same. Factors such as fluid residence times, shear stress, and liquid-to-cell volume ratios can be adjusted so that they resemble those seen in vivo. Attempts to re-create the three-dimensionality of the cell culture environment have been made and need to be pursued further. The most important aspect in further facilitating the acceptance of μCCAs is to test the validity of the results obtained with μCCAs by comparing them with in vivo data under identical conditions and drug exposure. Such experiments will let us draw conclusions about how μCCAs could be used to enhance efficacy and toxicity predictions.

DISCLOSURE STATEMENT

M.L.S. is a member of the Scientific Advisory Board of Hurel Corp., which is commercializing body-on-a-chip devices.

ACKNOWLEDGMENTS

Work on this article was supported in part by the Nanobiotechnology Center (NBTC, project CM-2) and by the U.S. Army Corps of Engineers (CERL, grant W9132T-07).

LITERATURE CITED

1. Adams CP, Brantner VV. 2006. Estimating the cost of new drug development: Is it really 802 million dollars? *Health. Aff.* (*Millwood*) 25:420–28
2. Hughes B. 2010. 2009 FDA drug approvals. *Nat. Rev. Drug Discov.* 9:89–92
3. Kola I, Landis J. 2004. Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3:711–15
4. Booth B, Glassman R, Ma P. 2003. Oncology's trials. *Nat. Rev. Drug Discov.* 2:609–10
5. Lahoz A, Gombau L, Donato MT, Castell JV, Gomez-Lechon MJ. 2006. In vitro ADME medium/high-throughput screening in drug preclinical development. *Mini Rev. Med. Chem.* 6:1053–62
6. Carlson TJ, Fisher MB. 2008. Recent advances in high throughput screening for ADME properties. *Comb. Chem. High Throughput Screen.* 11:258–64

7. Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, et al. 1989. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J. Natl. Cancer Inst.* 81:1088–92
8. Hall LA, Krauthauser CM, Wexler RS, Hollingshead MG, Slee AM, Kerr JS. 2000. The hollow fiber assay: continued characterization with novel approaches. *Anticancer Res.* 20:903–11
9. Fiebig HH, Berger DP, Winterhalter BR, Plowman J. 1990. In vitro and in vivo evaluation of US-NCI compounds in human tumor xenografts. *Cancer Treat. Rev.* 17:109–17
10. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, et al. 2001. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. *Br. J. Cancer* 84:1424–31
11. Khetani SR, Szulgit G, Del Rio JA, Barlow C, Bhatia SN. 2004. Exploring interactions between rat hepatocytes and nonparenchymal cells using gene expression profiling. *Hepatology* 40:545–54
12. Takayama G, Taniguchi A, Okano T. 2007. Identification of differentially expressed genes in hepatocyte/endothelial cell co-culture system. *Tissue Eng.* 13:159–66
13. Bhatia SN, Balis UJ, Yarmush ML, Toner M. 1999. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *FASEB J.* 13:1883–900
14. Sung JH, Shuler ML. 2010. In vitro microscale systems for systematic drug toxicity study. *Bioprocess Biosyst. Eng.* 33:5–19
15. Li AP, Bode C, Sakai Y. 2004. A novel in vitro system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast cancer cells. *Chem. Biol. Interact.* 150:129–36
16. Ma B, Zhang G, Qin J, Lin B. 2009. Characterization of drug metabolites and cytotoxicity assay simultaneously using an integrated microfluidic device. *Lab Chip* 9:232–38
17. Vozzi F, Heinrich JM, Bader A, Ahluwalia AD. 2009. Connected culture of murine hepatocytes and HUVEC in a multicompartmental bioreactor. *Tissue Eng. Part A* 15:1291–99
18. Zhang C, Zhao Z, Abdul Rahim NA, van Noort D, Yu H. 2009. Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. *Lab Chip* 9:3185–92
19. Galbraith CG, Skalak R, Chien S. 1998. Shear stress induces spatial reorganization of the endothelial cell cytoskeleton. *Cell Motil. Cytoskelet.* 40:317–30
20. Fernandez P, Bourget C, Bareille R, Daculsi R, Bordenave L. 2007. Gene response in endothelial cells cultured on engineered surfaces is regulated by shear stress. *Tissue Eng.* 13:1607–14
21. Sin A, Chin KC, Jamil MF, Kostov Y, Rao G, Shuler ML. 2004. The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. *Biotechnol. Prog.* 20:338–45
22. Sung JH, Esch MB, Shuler ML. 2010. Integration of in silico and in vitro platforms for pharmacokinetic-pharmacodynamic modeling. *Expert Opin. Drug Metab. Toxicol.* 6:1063–81
23. Tatosian DA, Shuler ML. 2009. A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. *Biotechnol. Bioeng.* 103:187–98
24. Sung JH, Shuler ML. 2009. A micro cell culture analog (microCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. *Lab Chip* 9:1385–94
25. Viravaidya K, Shuler ML. 2004. Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies. *Biotechnol. Prog.* 20:590–97
26. Viravaidya K, Sin A, Shuler ML. 2004. Development of a microscale cell culture analog to probe naphthalene toxicity. *Biotechnol. Prog.* 20:316–23
27. Xu H, Kraus WL, Shuler ML. 2008. Development of a stable dual cell-line GFP expression system to study estrogenic endocrine disruptors. *Biotechnol. Bioeng.* 101:1276–87
28. Chen XJ, West AC, Cropek DM, Banta S. 2008. Detection of the superoxide radical anion using various alkanethiol monolayers and immobilized cytochrome *c*. *Anal. Chem.* 80:9622–29
29. Ges IA, Baudenbacher F. 2010. Enzyme-coated microelectrodes to monitor lactate production in a nanoliter microfluidic cell culture device. *Biosens. Bioelectron.* 26:828–33
30. Xing JZ, Zhu L, Gabos S, Xie L. 2006. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. *Toxicol. in Vitro* 20:995–1004

31. Xiao C, Luong JHT. 2005. Assessment of cytotoxicity by emerging impedance spectroscopy. *Toxicol. Appl. Pharmacol.* 206:102–12
32. Xiao C, Luong JHT. 2003. On-line monitoring of cell growth and cytotoxicity using electric cell-substrate impedance sensing (ECIS). *Biotechnol. Prog.* 19:1000–5
33. Curtis TM, Widder MW, Brennan LM, Schwager SJ, van der Schalie WH, et al. 2009. A portable cell-based impedance sensor for toxicity testing of drinking water. *Lab Chip* 9:2176–83
34. Male KB, Lachance B, Hrapovic S, Sunahara G, Luong JHT. 2008. Assessment of cytotoxicity of quantum dots and gold nanoparticles using cell-based impedance spectroscopy. *Anal. Chem.* 80:5487–93
35. Asphahani F, Zhang M. 2007. Cellular impedance biosensors for drug screening and toxin detection. *Analyst* 132:835–41
36. Tatosian DA, Shuler ML, Kim D. 2005. Portable in situ fluorescence cytometry of microscale cell-based assays. *Opt. Lett.* 30:1689–91
37. Oh TI, Sung JH, Tatosian DA, Shuler ML, Kim D. 2007. Real-time fluorescence detection of multiple microscale cell culture analog devices in situ. *Cytometry A* 71:857–65
38. Sung JH, Choi JR, Kim D, Shuler ML. 2009. Fluorescence optical detection in situ for real-time monitoring of cytochrome P450 enzymatic activity of liver cells in multiple microfluidic devices. *Biotechnol. Bioeng.* 104:516–25
39. Malet-Martino M, Martino R. 2002. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. *The Oncologist* 7:288–323
40. Borowski E, Bontemps-Gracz MM, Piwkowska A. 2005. Strategies for overcoming ABC-transporters-mediated multidrug resistance (MDR) of tumor cells. *Acta Biochim. Pol.* 52:609–27
41. Pascaud C, Garrigos M, Orlowski S. 1998. Multidrug resistance transporter P-glycoprotein has distinct but interacting binding sites for cytotoxic drugs and reversing agents. *Biochem. J.* 333(Pt. 2):351–58
42. Lehnert M, Dalton WS, Roe D, Emerson S, Salmon SE. 1991. Synergistic inhibition by verapamil and quinine of P-glycoprotein-mediated multidrug resistance in a human myeloma cell line model. *Blood* 77:348–54
43. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. 2000. Role of quinones in toxicology. *Chem. Res. Toxicol.* 13:135–60
44. Tsuruda LS, Lame MW, Jones AD. 1995. Formation of epoxide and quinone protein adducts in B6C3F1 mice treated with naphthalene, sulfate conjugate of 1,4-dihydroxynaphthalene and 1,4-naphthoquinone. *Arch. Toxicol.* 69:362–67
45. Quick DJ, Shuler ML. 1999. Use of in vitro data for construction of a physiologically based pharmacokinetic model for naphthalene in rats and mice to probe species differences. *Biotechnol. Prog.* 15:540–55
46. Buonarati M, Morin D, Plopper C, Buckpitt A. 1989. Glutathione depletion and cytotoxicity by naphthalene 1,2-oxide in isolated hepatocytes. *Chem. Biol. Interact.* 71:147–65
47. Buckpitt A, Chang AM, Weir A, Van Winkle L, Duan X, et al. 1995. Relationship of cytochrome P450 activity to Clara cell cytotoxicity: IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. *Mol. Pharmacol.* 47:74–81
48. Richieri PR, Buckpitt AR. 1988. Glutathione depletion by naphthalene in isolated hepatocytes and by naphthalene oxide in vivo. *Biochem. Pharmacol.* 37:2473–78
49. Wilson AS, Davis CD, Williams DP, Buckpitt AR, Pirmohamed M, Park BK. 1996. Characterization of the toxic metabolite(s) of naphthalene. *Toxicology* 114:233–42
50. Flowers-Geary L, Bleczinki W, Harvey RG, Penning TM. 1996. Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon ortho-quinones produced by dihydrodiol dehydrogenase. *Chem. Biol. Interact.* 99:55–72
51. Kilanowicz A, Czerski B, Sapota A. 1999. The disposition and metabolism of naphthalene in rats. *Int. J. Occup. Med. Environ. Health* 12:209–19
52. Palackal NT, Burczynski ME, Harvey RG, Penning TM. 2001. The ubiquitous aldehyde reductase (AKR1A1) oxidizes proximate carcinogen *trans*-dihydrodiols to *o*-quinones: Potential role in polycyclic aromatic hydrocarbon activation. *Biochemistry* 40:10901–10
53. Buck Louis GM, Lynch CD, Cooney MA. 2006. Environmental influences on female fecundity and fertility. *Semin. Reprod. Med.* 24:147–55

54. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, et al. 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ. Health Perspect.* 113:1056–61
55. Yang M, Ryu JH, Jeon R, Kang D, Yoo KY. 2009. Effects of bisphenol A on breast cancer and its risk factors. *Arch. Toxicol.* 83:281–85
56. Bitman J, Cecil HC, Harris SJ, Fries GF. 1968. Estrogenic activity of o,p′-DDT in the mammalian uterus and avian oviduct. *Science* 162:371–72
57. Metcalf JL, Laws SC, Cummings AM. 1996. Methoxychlor mimics the action of 17β-estradiol on induction of uterine epidermal growth factor receptors in immature female rats. *Reprod. Toxicol.* 10:393–99
58. Harris CA, Henttu P, Parker MG, Sumpter JP. 1997. The estrogenic activity of phthalate esters in vitro. *Environ. Health Perspect.* 105:802–11
59. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, et al. 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. *Environ. Health Perspect.* 111:1180–87
60. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. 1995. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environ. Health Perspect.* 103:582–87
61. Kumar V, Chambon P. 1988. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 55:145–56
62. Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, et al. 2000. Estrogen receptor pathways to AP-1. *J. Steroid Biochem. Mol. Biol.* 74:311–17
63. Warner M, Nilsson S, Gustafsson JA. 1999. The estrogen receptor family. *Curr. Opin. Obstet. Gynecol.* 11:249–54
64. Lee HJ. 2002. Protein drug oral delivery: the recent progress. *Arch. Pharm. Res.* 25:572–84
65. Lampen A, Bader A, Bestmann T, Winkler M, Witte L, Borlak JT. 1998. Catalytic activities, protein- and mRNA-expression of cytochrome P450 isoenzymes in intestinal cell lines. *Xenobiotica* 28:429–41
66. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* 13:407–84
67. Lau YY, Chen YH, Liu TT, Li C, Cui X, et al. 2004. Evaluation of a novel in vitro caco-2 hepatocyte hybrid system for predicting in vivo oral bioavailability. *Drug Metab. Dispos.* 32:937–42
68. Choi S, Nishikawa M, Sakoda A, Sakai Y. 2004. Feasibility of a simple double-layered coculture system incorporating metabolic processes of the intestine and liver tissue: application to the analysis of benzo[a]pyrene toxicity. *Toxicol. In Vitro* 18:393–402
69. Brand RM, Hannah TL, Mueller C, Cetin Y, Hamel FG. 2000. A novel system to study the impact of epithelial barriers on cellular metabolism. *Ann. Biomed. Eng.* 28:1210–17
70. Choi SH, Fukuda O, Sakoda A, Sakai Y. 2004. Enhanced cytochrome P450 capacities of Caco-2 and Hep G2 cells in new coculture system under the static and perfused conditions: evidence for possible organ-to-organ interactions against exogenous stimuli. *Mater. Sci. Eng. C* 24:333–39
71. Mahler GJ, Esch MB, Glahn RP, Shuler ML. 2009. Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity. *Biotechnol. Bioeng.* 104:193–205
72. Mahler GJ, Shuler ML, Glahn RP. 2009. Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. *J. Nutr. Biochem.* 20:494–502
73. Gujral JS, Knight TR, Farhood A, Bajt ML, Jaeschke H. 2002. Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? *Toxicol. Sci.* 67:322–28
74. Mondrinos MJ, Koutzaki S, Jiwanmall E, Li M, Dechadarevian JP, et al. 2006. Engineering three-dimensional pulmonary tissue constructs. *Tissue Eng.* 12:717–28
75. Maina JN, West JB. 2005. Thin and strong! The bioengineering dilemma in the structural and functional design of the blood-gas barrier. *Physiol. Rev.* 85:811–44
76. Nalayanda DD, Puleo CM, Fulton WB, Wang TH, Abdullah F. 2007. Characterization of pulmonary cell growth parameters in a continuous perfusion microfluidic environment. *Exp. Lung Res.* 33:321–35
77. Bilek AM, Dee KC, Gaver DP III. 2003. Mechanisms of surface-tension-induced epithelial cell damage in a model of pulmonary airway reopening. *J. Appl. Physiol.* 94:770–83
78. Huh D, Fujioka H, Tung YC, Futai N, Paine R III, et al. 2007. Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. *Proc. Natl. Acad. Sci. USA* 104:18886–91

79. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. 2010. Reconstituting organ-level lung functions on a chip. *Science* 328:1662–68
80. Ingber DE. 2006. Cellular mechanotransduction: putting all the pieces together again. *FASEB J.* 20:811–27
81. Grieb P, Forster RE, Strome D, Goodwin CW, Pape PC. 1985. O_2 exchange between blood and brain tissues studied with $^{18}O_2$ indicator-dilution technique. *J. Appl. Physiol.* 58:1929–41
82. Pardridge WM, Triguero D, Yang J, Cancilla PA. 1990. Comparison of in vitro and in vivo models of drug transcytosis through the blood-brain barrier. *J. Pharmacol. Exp. Ther.* 253:884–91
83. Levin VA. 1980. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J. Med. Chem.* 23:682–84
84. Ohno K, Pettigrew KD, Rapoport SI. 1978. Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. *Am. J. Physiol.* 235:H299–307
85. Garberg P, Ball M, Borg N, Cecchelli R, Fenart L, et al. 2005. In vitro models for the blood-brain barrier. *Toxicol. In Vitro* 19:299–334
86. Bauer HC, Bauer H. 2000. Neural induction of the blood-brain barrier: still an enigma. *Cell. Mol. Neurobiol.* 20:13–28
87. Davson H, Oldendorf WH. 1967. Symposium on membrane transport: transport in the central nervous system. *Proc. R. Soc. Med.* 60:326–29
88. Ma SH, Lepak LA, Hussain RJ, Shain W, Shuler ML. 2005. An endothelial and astrocyte co-culture model of the blood-brain barrier utilizing an ultra-thin, nanofabricated silicon nitride membrane. *Lab Chip* 5:74–85
89. Gumbleton M, Audus KL. 2001. Progress and limitations in the use of in vitro cell cultures to serve as a permeability screen for the blood-brain barrier. *J. Pharm. Sci.* 90:1681–98
90. Wilkening S, Stahl F, Bader A. 2003. Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties. *Drug Metab. Dispos.* 31:1035–42
91. Chia SM, Lin PC, Yu H. 2005. TGF-β1 regulation in hepatocyte-NIH3T3 coculture is important for the enhanced hepatocyte function in 3D microenvironment. *Biotechnol. Bioeng.* 89:565–73
92. Zhang C, Chia SM, Ong SM, Zhang S, Toh YC, et al. 2009. The controlled presentation of TGF-β1 to hepatocytes in a 3D-microfluidic cell culture system. *Biomaterials* 30:3847–53
93. Sung JH, Kam C, Shuler ML. 2010. A microfluidic device for a pharmacokinetic-pharmacodynamic (PK-PD) model on a chip. *Lab Chip* 10:446–55
94. Novik E, Maguire TJ, Chao P, Cheng KC, Yarmush ML. 2010. A microfluidic hepatic coculture platform for cell-based drug metabolism studies. *Biochem. Pharmacol.* 79:1036–44
95. Xu H, Shuler ML. 2009. Quantification of chemical-polymer surface interactions in microfluidic cell culture devices. *Biotechnol. Prog.* 25:543–51
96. Chao P, Maguire TJ, Novik E, Cheng KC, Yarmush ML. 2009. Evaluation of a microfluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human. *Biochem. Pharmacol.* 78:625–32
97. Sin A, Reardon CF, Shuler ML. 2004. A self-priming microfluidic diaphragm pump capable of recirculation fabricated by combining soft lithography and traditional machining. *Biotechnol. Bioeng.* 85:359–63

Annual Review of Biomedical Engineering
Volume 13, 2011

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Biomedical Engineering* articles may be found at http://bioeng.annualreviews.org/