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How multi-organ microdevices can help foster drug development ☆

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ABSTRACT

Multi-organ microdevices can mimic tissue–tissue interactions that occur as a result of metabolite travel from one tissue to other tissues in vitro. These systems are capable of simulating human metabolism, including the conversion of a pro-drug to its effective metabolite as well as its subsequent therapeutic actions and toxic side effects. Since tissue–tissue interactions in the human body can play a significant role in determining the success of new pharmaceuticals, the development and use of multi-organ microdevices present an opportunity to improve the drug development process. The devices have the potential to predict potential toxic side effects with higher accuracy before a drug enters the expensive phase of clinical trials as well as to estimate efficacy and dose response. Multi-organ microdevices also have the potential to aid in the development of new therapeutic strategies by providing a platform for testing in the context of human metabolism (as opposed to animal models). Further, when operated with human biopsy samples, the devices could be a gateway for the development of individualized medicine. Here we review studies in which multi-organ microdevices have been developed and used in a ways that demonstrate how the devices' capabilities can present unique opportunities for the study of drug action. We will also discuss challenges that are inherent in the development of multi-organ microdevices. Among these are how to design the devices, and how to create devices that mimic the human metabolism with high authenticity. Since single organ devices are testing platforms for tissues that can later be combined with other tissues within multi-organ devices, we will also mention single organ devices where appropriate in the discussion.

Contents

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1. Introduction

1.1. Limitations of the current drug development process

Modern drug development requires implementation of extensive pre-clinical testing and validation protocols before potential therapeutic compounds are approved to progress to clinical evaluation. This process is costly and time-consuming, as well as inefficient as for every ten drugs entering clinical trials, only one or two will typically be licensed for eventual use in humans [1,2].

One of the major factors influencing this poor success rate is the lack of preclinical model systems capable of providing accurate predictions of human responses to novel therapeutic drugs. The current gold standard for laboratory based preclinical evaluation is a combination of in vitro cell culture assay and in vivo animal model experimentation and assessment. Cell culture assays are advantageous since they provide controlled environments where cellular maturation and activity are easily observed and tested. However, cultures of single cell types, or even co-cultures of 2 or 3 complimentary cell types, lack the complexity of living systems and are incapable of modeling situations where organ–organ or tissue–tissue communication is important. This simplicity is a major drawback in drug development studies since it is difficult to predict the oftentimes complex drug metabolism and the effect of metabolite activity on non-target tissues. Moreover, cells maintained in standard in vitro culture conditions often suffer from incomplete maturation, or are held in a configuration that prevents their full functional development, making predictions of in vivo tissue function more difficult to extrapolate.

Animal models maintain the intricacy of living systems, making assessment of organ–organ crosstalk and non-target organ toxicity possible. However, the inherent complexity of interconnected tissues can make specific modes of action difficult to elucidate and therefore confound observations. Furthermore, animal models have, on multiple occasions, been demonstrated to be poor predictors of human responses to drug treatment. The assumption that beneficial outcomes observed in animals will translate to human patients has led to clinical situations where treatments have proved ineffective or even harmful to patient wellbeing and recovery [3].

Many of the current in vitro models used by the pharmaceutical industry consist of isolated single cells from a single organ. This simplification does not reflect the complexity of the organ's interaction that occurs with the rest of the body in vivo. Indeed, it is well recognized that toxicity phenomena are a consequence of a complex series of events that can involve several organs. For example, bioactivation of a drug by specific liver enzymes may result in toxic events at a different organ. The current limitations of experimental methods confirm the need of an intermediate human in vitro model in the early stage of drug development, a model that could efficiently reproduce multi-organ interactions to better predict the side effects observed in clinical trials.

The development of more appropriate and informative human models for preclinical drug screening would improve the success rate of clinical trials. Models that could provide predictions with higher accuracy would reduce the cost of therapeutic development and improve the speed at which new drugs are approved for patients. They could also reduce or ultimately eliminate the number of animal experiments needed and thereby reduce ethical concerns. To this end, recent research efforts have focused on establishing physiologically relevant, multi-organ, functional in vitro models utilizing human cell sources. Such models are currently being designed to promote full functionality and molecular maturation of human cell types in configurations that facilitate the measurement and assessment of cell performance in real-time and in a high-throughput manner.

1.2. The concept of multi-organ microdevices

Multi-organ microdevices are microfluidic devices that mimic key aspects of human metabolism by connecting the fluidic streams from several on-chip in vitro tissue cultures with each other in a physiologically relevant manner so that metabolites are consumed, produced, and exchanged (via recirculation) between all tissues at physiologically relevant concentrations. The devices have been referred to as micro-cell culture analogs (μCCA), microphysiological systems, or multi-organ microsystems. The combination of several tissues allows one to represent the function of several organs and observe their individual response to a drug as well as the influence this response exerts on other organs. Multi-organ devices can be used to simulate the conversion of a pro-drug to an effective compound that acts on another tissue as well as the compound's toxicity at tissues that are not the intended target tissue [4,5]. The ability of some tissues to modulate drug toxicity – for example, fat tissue, which can ameliorates the toxicity by storing compounds and thereby reducing its concentration within the fluidic stream – has been observed [6,7]. The devices can also shed light on the quantitative influence that barrier tissues such as skin, lung epithelium, gastrointestinal tract epithelium, and endothelium impose on the bioavailability of a drug at the intended target tissue [8–11]. Within the device any number of tissues can be connected and the circulation of soluble metabolites between them enables the simulation and prediction of tissue–tissue interactions that are important in drug development [12,13].

While tissue–tissue interactions can be simulated with static cell culture systems [14], the use of microtechnology allows chamber sizes and fluidic circuitry to be designed in a way that makes the simulation more physiologically relevant (Fig. 1). For example, organ chamber sizes can be designed so that fluid residence times within them relate to each other as they do in vivo. According to this approach, a kidney chamber would be a fraction of the size of a liver chamber. In addition, the sizes of the connecting fluid channels can be designed to distribute the blood surrogate according to the in vivo blood distribution. For example, the circulation in the body delivers similar amounts of blood to the kidney and the liver despite their difference in size [15,16]. Other advantages of microfabrication include the ability to fabricate many devices in a cost-effective manner. In addition, many cells/tissues respond to mechanical forces, such as those derived from fluid flow, and the cell's response to mechanical forces can be mimicked in a microfabricated microfluidic system. The redesign and implementation of changes/improvements of the devices can be done fairly rapidly. Microfabrication techniques allow for the precise implementation of design principles while allowing for flexibility at a relatively low cost.

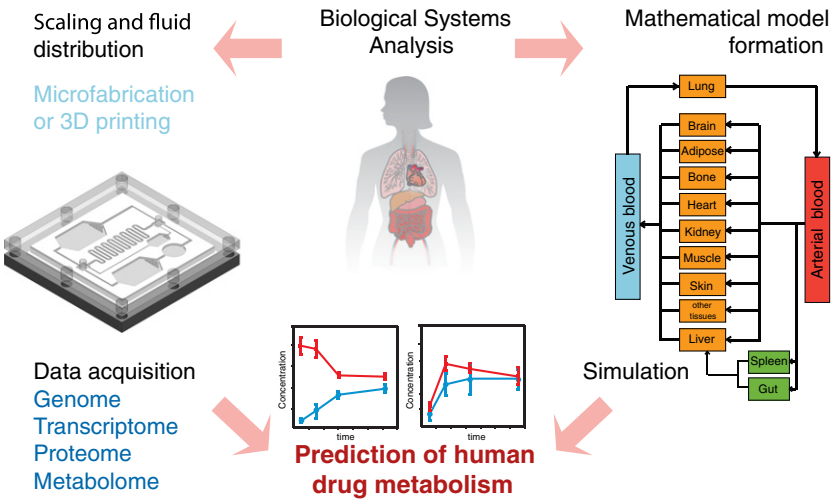

Fig. 1. Biological system analysis is the basis for both PBPK model design and multi-organ microdevice design. Results obtained from simulations of drug action with either system can be compared with each other. Discrepancies must be resolved and the resulting knowledge can then inform next generation models.

The principles guiding the development of physiologically based pharmacokinetic (PBPK) models provide guidance for designing multi-organ microdevices (Fig. 1). In PBPK models, every organ is represented as a compartment. The uptake, distribution, metabolism, and excretion (ADME) of a drug is described with a set of differential equations. Multi-organ microdevices can be seen as physical representations of PBPK models, in which the organs are represented by an actual compartment, and the equations that govern the ADME of a drug are physically carried out by the tissues and fluidic channels that connect the organ chambers. This strategy led to the classification of such devices as cell culture analogs of PBPKs, and later as the micro-cell culture analogs of PBPKs (μCCAs) [17]. Using this idea, one may design a device by first building a PBPK model that contains the organs of interest and then build a physical device that corresponds to the mathematical model. For example, Fig. 2 shows visually how the PBPK of the human body can be simplified by considering organs of interest explicitly but combining slowly perfused tissues and rapidly perfused tissues into two separate compartments. Combining organs is a valid approach when these organs neither react with nor absorb the drug or its metabolites and hence the organs are not important to capturing the full dynamics of drug and metabolite distribution. In the example given in Fig. 2, the gastrointestinal tissue, bone marrow, adipose tissue, kidney, and liver tissue were considered explicitly, while the other tissues were combined. Additionally, depending on the question that is to be addressed with the device, fluid pathways mimicking blood recirculation (expressed in the model as arrows) may be simplified as well. In a setup in which the fluid is split passively, the dimensions of the fluid channels determine how much fluid flows to each organ chamber. While the organ chamber sizes are scaled by a factor (typical scaling ranges from 40,000 to 250,000), the percentage of fluid flow that reaches each organ chamber should be comparable to the percent blood flow the respective organ receives in vivo. Through adjusting the pressure drop across each of the channels by adjusting the channel dimensions, the needed fluid flow rate can be achieved. Of course, the fewer simplifications are made to the PBPK, the more authentic will be the device's response to a drug. The complexity of the device should be sufficient to answer the question of interest, but a full model of the human body may not always be necessary. An advantage that arises from this approach is that

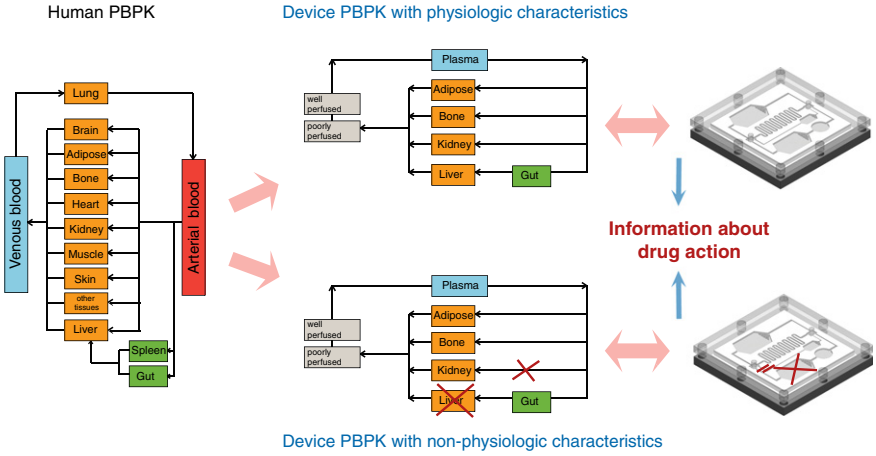

Fig. 2. Illustration of how simplified PBPK models can guide the design of multi-organ microdevices: Organs that are of interest for a particular study are incorporated as chambers on the device. Organs that are expected to interact with the drug can be combined within one volume (filled with liquid for well-perfused organs or filled with hydrogel for poorly perfused organs). The arrows in the schematic representation of the mathematical model are represented as fluidic circuitry on the multi-organ platform. The physiologically designed device that is illustrated here (top) was used for the simulation of the first pass metabolism of acetaminophen [8]. The devices can also be operated in non-physiologic modes without certain organs, or with limited fluid flow to organs (bottom illustration) to obtain information about the mechanisms of drug action.

the results of the PBPK and the physical device can be compared and any discrepancies may lead to opportunities of further investigation and a deeper understanding of the biological system. PBPKs provide a powerful approach to designing multi-organ devices in a physiologically relevant manner.

1.3. Multi-organ microdevices versus animal models

Multi-organ microdevices have several advantages over the use of animal models for drug testing, with the most important one being that the devices can be operated with human cells and hence they are capable of mimicking the human metabolism instead of animal metabolism. When scaled appropriately (according to organ sizes and considering the cell density and activity in the in vitro tissue constructs) and when operated with a physiologic ratio of blood surrogate to cells, the metabolite concentrations in the device are the same as in the human body and predictions of effective and toxic concentrations can be made. This is not always possible when extrapolating data from animal models to humans.

Additionally, the devices are more cost effective and allow for the testing of combinations of compounds at varying concentrations when operating many of them in parallel. The number of animals that would be needed for such screening would be rather high and add to the costs of drug development as well as to the ethical concerns that accompany drug testing with animals.

Another important distinction from animal models is that multi-organ microdevices can be operated using physiologic and non-physiologic conditions (Fig. 2). The use of physiologic conditions for predicting drug action is obvious, but the use of non-physiologic conditions can be of advantage as well. For example, if the origin of a toxic metabolite is unclear, some of the organs within the device could be set up with smaller or larger volumes than would be physiologic (or they could be left out entirely). The dose response information resulting from experiments with such devices can give important information about drug action.

Experimental results from experiments with multi-organ microdevices can be compared to predictions obtained with mathematical PBPK models. These mathematical models can be tailored to reflect the constellation of the on-chip situation. Since mathematical models rely on our knowledge of metabolic pathways, any discrepancies between the data from the models and experiments point to a gap in our understanding of the human metabolism. Additional further investigations will likely expand our knowledge and understanding, which can in turn be incorporated into the model.

Multi-organ microdevices also provide paths to individualized medicine when operated with patient biopsy samples, and for investigating new therapeutic strategies that could bear high risks, but have a potential for high impact.

1.4. Single organ microdevices

Microfluidic single organ models provide advantages over static cultures, and have been used to investigate disease states and drug-action. Since the liver is the most important organ for drug metabolism and clearance, many efforts have been dedicated to developing liver models that function well within microfluidic devices [18–30]. On chip tissues of the gastrointestinal tract [31–34], the skin [35,36], lung [37–39], heart [40,41], microvasculature [42–45], and kidney [46–49] have also been developed. Since we are focusing our attention on multi-organ devices that demonstrate a successful operation with at least two organs or tissues within one device, the discussion of single organ devices will not be exhaustive. For a more complete review of single organ tissues, we refer the reader to review articles that focus on single organ microdevices [50,51,12,13,17,52–54].,

2. Examples of multi-organ microdevices and ways in which they can contribute to the drug development process

2.1. Lowering the cost of drug discovery

The drug development process is expensive, especially in the phases of clinical trials, which can cost billions of dollars. However, despite extensive animal testing of drugs before the start of a clinical trial with humans, many drugs fail, because low efficacy and toxic side effects were not predicted accurately. This highlights the fact that animal and human metabolism are different. The most promising advantage of multi-organ microdevices is that they can mimic both animal and human metabolism and predict differences between them when evaluating drugs. This will allow for a higher level of accuracy when predicting the outcome of clinical trials. Accurately predicting toxic side effects can prevent unsuitable drug candidates from entering the expensive phase of clinical trials and limit costs and unrealistic expectations.

Multi-organ microdevices can also reduce the cost of drug testing because they provide low cost platforms for the evaluation of many chemicals and combinations of chemicals. Using these devices, it is possible to test more drug formulations at a low cost and then eliminate ineffective or toxic concentrations from the parameter space that would be tested with animals. In order to predict effective drug concentrations, the devices must be designed in a way such that they produce similar concentrations of metabolites as are found in the human body. This is not impossible but can be challenging considering that cellular activity and density of an in vitro construct can differ considerably from those of in vivo tissues. Several groups have published their efforts towards developing multi-organ microdevices (Table 1). Below we discuss these studies, focussing on the devices' potential use in drug testing applications.

2.1.1. Predicting drug efficacy and toxic side effects for humans

The first step to demonstrate that multi-organ microdevices can predict efficacy and drug toxicity is to build devices with a limited number of key organs and to challenge them with a drug whose efficacy and toxicity are known and can be measured in the device. Here we discuss two studies in which this has been achieved with known chemotherapeutics.

Liver and kidney tissues are of great interest to drug developers due to their predominant role during the absorption, distribution, metabolism, and excretion (ADME) process of a drug. Physiologically, the liver is the main organ in which the metabolism of drugs occurs, while the kidney is involved in their elimination. These critical processes make these two organs highly susceptible to drug injury. Experiments with the anti-cancer drug ifosfamide illustrate the importance of the liver–kidney interaction. Ifosfamide is a prodrug that is bioactivated by CYP450 enzymes in the liver. The generated metabolites have efficient anti-tumor effects but some of them, such as chloroacetaldehyde, are recognized to be nephrotoxic. This mechanism was simulated in the first liver–kidney co-culture microchip [55]. When the authors used highly differentiated liver cell models (HepaRG), they were able to measure a perturbation of cell proliferation and calcium release in the kidney tissue in response to the drug. These results were not observed when the device did not contain liver cells or when the liver cell line HepG2/C3a (known to express lower levels of CYP450 enzymes than hepatocytes in the liver) was used. This contribution demonstrates the relevance of multi-organ interactions in drug testing and also highlights the importance of the cell source when conducting toxicological studies.

Another system that demonstrated the ability of multi-organ microdevices to simulate the exchange of metabolites between organs was a system that contained liver cells (HepG2/C3A), colon cancer cells (HCT-116), and myeloblasts (Kasumi-1) [5]. The device was subjected to Tegafur, an oral prodrug of the anti-cancer drug, 5-fluourouracil (5-FU). Upon oral uptake of 5-FU the enzyme

Table 1
Examples of multi-organ microdevices and their demonstrated capabilities for drug testing applications.

Organ combination	Tested drug/toxin or other challenge	Demonstrated capability	Endpoints	References
GI-tract–liver	Acetaminophen, 7-ethoxycoumarin (7-EC), 7-hydroxycoumarin (7-HC) and lidocaine	Metabolism of acetaminophen in the GI-tract and liver, modulated acetaminophen toxicity in the liver due to the presence of the GI-tract, Modulation of liver toxicity due to addition of bile acids to GI-tract model	Acetaminophen metabolite detection via HPLC, live/dead assay, P450 7A1 (CYP7A1) activity in the liver	[8,9,61]
Microvasculature–liver	Not challenged with any drugs	Increased albumin and urea production as a result of co-culture	Consumption of glucose by liver cells, albumin synthesis, urea and lactate production	[63–66]
Microvasculature–tumor	Macrophages Fibrosarcoma cells	Barrier function to tumor cell invasion	Number of tumor cell and endothelial cell interactions	[114]
Liver–tumor–bone marrow	Combinations of doxorubicin with cyclosporine and nicardipine Tegafur	Synergistic drug action Pro-drug conversion, synergy of anti-cancer drugs	Live/dead assay	[4,5]
Liver–lung–fat	Naphthalene	Identification of toxic metabolite, modulation of toxicity by fat tissue	Live/dead assay	[6,7]
Liver–kidney	Ifosfamide	Pro-drug conversion and nephrotoxicity	Metabolite detection via mass spectrometry, cell proliferation, calcium release	[55]
Liver–lung–kidney–fat	TGF-ß1	Dose-dependent response of each cell type to TGF-ß1 and tissue-specific support through localization of TGF-ß1 release	Albumin secretion, PROD activity, GGT activity, adiponectin secretion	[78]
Skin–liver	bpV(phen)	Uptake of bpV(phen) through the skin and subsequent stimulation of glucose consumption	Glucose consumption	[60,10]

dihydropyrimidine dehydrogenase (DPD) rapidly degrades the drug, resulting in a low effective concentration [56]. Administration of the prodrug Tegafur, which is converted to 5-FU by the P450 1A2, 2A6, and 2C8 enzymes [57], instead of administering 5-FU directly to patients has advantages because the uptake of Tegafur results in more stable concentrations of the effective component 5-FU. Challenging the multi-organ device with Tegafur, the metabolism of Tegafur, i.e. its conversion to 5-FU in the liver cell compartment was reproduced. 5-FU traveled through the microfluidic channels to the cancer cell compartment, where it caused a dose-dependent decrease in cell viability. Clinically Tegafur is often given with uracil, a competitive inhibitor of 5-FU, which slows its degradation and thereby increases the circulation time of 5-FU. The multi-organ system demonstrated that uracil addition did result in increased effectiveness of Tegafur (increased cell death) and that the optimal ratio in vitro (about 4 mol of uracil to 1 mol of Tegafur) corresponds to the clinically determined optimum [5].

2.1.2. Predicting the bioavailability of drugs

The rate of first pass metabolism of ingested drugs (due to the direct transport to the liver via the portal circulation) and the distribution of its metabolites via the systemic circulation determine the drug's bioavailability. Predicting the bioavailability of a drug accurately can be difficult with animal models. Multi-organ microdevices that contain a combination of the gastrointestinal tract epithelium and the liver at the appropriate sizes and with realistic liquid to cell ratios have the potential to predict the bioavailability of ingested drugs. To simulate the first pass metabolism of ingested drugs in conventional static models, Caco-2 cells are typically grown on porous membranes. The cells are in contact with hepatocytes that are grown in a wells beneath the membrane via a common medium [30,58,59] Substances that are transported across the Caco-2 cell layer and metabolites that are generated in the Caco-2 culture can reach the liver cell culture and vice versa. Microfluidic models of the liver [18–29] can be combined with models of the GI-tract epithelium. In fluidic models of the first pass metabolism, fluidic circuitry transports any substances that crossed the epithelial cell layer or that were generated by it from the basolateral side of the GI-tract cell culture to the liver cells that are located downstream [60]. Re-circulation of medium between the basolateral side of the GI-tract cell culture and the liver cell culture has also been implemented [8,61]. Using such models, the first pass metabolism of drugs such as acetaminophen has been simulated [8]. The results indicate that liver cell damage occurs in a dose-dependant manner. Since acetaminophen is a small non-ionized molecule that diffuses passively across the GI-tract epithelium, the presence of the GI-tract tissue presents a barrier to the drug, which in turn modulates the effects of drug concentration in the liver. The GI-tract epithelium exhibited modest P450 activity and converted a portion of acetaminophen into non-toxic metabolites. These metabolites were detected in the re-circulated medium using a high-performance liquid chromatography (HPLC). The result obtained with the first pass metabolism model was consistent with those obtained with acetaminophen challenges in mice [62]. Models of the first pass metabolism are capable of estimating its bioavailability and its considerable effect on a drug's toxicity to the liver.

A number of other barrier tissues can limit the bioavailability of drugs at the intended target tissue. Among these are the skin, the lung epithelium, the blood placental barrier, the blood brain barrier, and the endothelial lining of the microvasculature. Some of these barrier tissues have been incorporated within multi-organ microdevices [10,60]. For example, Brand et al. modified their model of the first pass metabolism to include a model of the skin instead of the GI-tract epithelium [60]. With these models, the topical application of drugs can be simulated. A number of other studies have addressed models of the liver or tumor tissues that contain some functionality of the microvasculature [21,63–66]. Models that include a microvascular

component will allow for the simulation of drugs that were intravenously administered in a more realistic fashion.

Another tissue that modulates the concentrations of substances that circulate within the blood stream is adipose tissue. This tissue can store and release chemicals depending on the degree of their hydrophobicity. This characteristic was demonstrated in 2004 with a multi-organ microdevice, with which the authors showed that the presence of a fat compartment altered the dynamics of naphthalene toxicity [6,7]. The addition of differentiated 3T3-L1 adipocytes to the device suggested that storage of naphthalene and naphthoquinone in fat tissue reduces glutathione depletion in the lung compartment, thus reducing the toxic effect. Generally, adipose tissue is an important tissue in modulating the concentration of a drug in the blood stream, but it is not often considered explicitly in multi-organ microdevices.

2.1.3. Testing combinations of drugs to elucidate synergistic drug action

Since microdevices are relatively inexpensive, and many such devices can be operated in parallel, it is possible to test numerous drugs and combinations of drugs at different concentrations. Testing combinations of drugs is useful when drug interactions may occur and synergistic interactions are of particular interest. Several drugs that have similar functions, but different side effects could potentially be combined at reduced dosages to achieve the needed tissue response. Such a response was seen with a system that contained liver tissue, tumor tissues, and bone marrow tissue [4]. Using this device, Tatosian et al. tested a combination of three drugs: a chemotherapeutic drug, doxorubicin, and two drugs that suppress multidrug resistance (MDR), nicardipine and cyclosporine. The two MDR drugs were chemical modulators that inhibit the action of the membrane transporter P-glycoprotein (P-gp) that pumps drugs out of the cell and prevents anti-cancer drugs from reaching sufficiently high intracellular concentrations to be effective as toxins for tumor cells [67]. Experiments with the uterine cancer cell line MES-SA, and an MDR variant of uterine cancer, MES-SA/DX-5, that overexpresses P-gp, indicated that combining the chemotherapeutic doxorubicin with the MDR modulators cyclosporine and nicardipine was 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. The device also contained megakaryoblast cells that form platelets (MEG-01) and liver cells (HepG2/C3A). Liver cells and bone marrow cells (MEG-01) were growth-inhibited when a single MDR modulator was used, but not when the combination of two modulators was used. 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 multi-organ microdevice. In clinical studies, when administered as single drugs, the high modulator concentration caused toxic side effects. The hypothesis that a combination of reduced doses of multiple modulators could be effectively combined with chemotherapeutics to reverse the growth of multi-drug resistant tumors with reduced side effects [68,69] was tested successfully with the multi-organ microdevice. Multi-drug combinations could potentially also be tested for particular patients that do not respond to routinely used drug combinations. Using biopsy cells within the devices could be one way to find individualized treatment options for these patients, estimating both drug efficacy and side effects. The possibility for individualized medicine is briefly discussed in Section 2.4.

2.2. Experimenting with non-physiologic versions of the human body

Multi-organ microdevices also present opportunities that animal models do not. For example, these devices can be operated with the entire set of organs of the human body, or with a subset. Leaving out an organ for a particular experiment or increasing its volume or activity beyond physiologic values can confirm or disprove hypotheses that aim to identify the origin of a toxic metabolite (Fig. 1). Further, it is possible to modify the devices to simulate disease conditions such as limited activity of cells, and limited blood supply to a particular organ. Below we describe studies that utilized this approach.

Low efficacy and the occurrence of toxic side effects are among the main reasons for drug attrition. Toxic metabolites can be generated from an initially non-toxic substance and these metabolites can circulate to other organs where they can cause substantial disturbances. Since multi-organ microdevices contain several tissues, the generation of toxic metabolites in any of these tissues and their actions on any of the other tissues within the device can be tested. Well-designed experimental sequences can even be used to test hypotheses about mechanisms of drug and chemical actions and this approach has been demonstrated with several devices. For example, a system that contained three tissues: liver, lung, and fat was challenged with naphthalene and the device response showed which organ was responsible for the generation of toxic metabolites and which metabolite was responsible for the cell death in another tissue [6,7,70]. Naphthalene is an environmental toxin, but we discuss it in this review, because it illustrates how multi-organ microdevices could be used to clarify mechanisms of drug action and toxicity. After naphthalene addition to the system, the liver formed reactive metabolites, which were released subsequently into the recirculating blood surrogate. The medium stream delivered the metabolites to the lung compartment, causing dose dependent lung cell death. Removing the liver cells and replacing them with lung cells or no cells at all allowed the authors to make the conclusion that the reactive metabolites of naphthalene were formed in the liver and not in the lung tissue. Increasing liver cell numbers caused an increase in toxicity in the lung compartment [70]. This result was consistent with the hypothesis that the reactive product of naphthalene metabolism in the liver was the cause of lung cell death. The result also suggests that the toxic compounds had a sufficient lifetime in the medium to be excreted and circulate to the lung compartment. Subsequent work using a multi-organ system demonstrated that the reactive metabolite was likely naphthoquinone rather than the naphthalene epoxide that had been previously proposed as the toxic compound [71]. This finding was confirmed using a microscale system with lung, liver and fat compartments [6,7]. This example suggests that several well-designed experiments with multi-organ microdevices can be used to determine which metabolite is responsible for a beneficial result within the target tissue.

2.3. Determining parameters for physiologically based pharmacokinetic models

Physiologically based pharmacokinetic models (PBPKs) are mathematical models that are used to extrapolate data from animal experiments and predict human response to a drug. The models rely on our understanding and knowledge of a drug's metabolism in order to give accurate predictions. Missing information means that the equations used in a PBPK are not sufficient and the model's predictive power is not as high as it could be if completed. Multi-organ microdevices can be modeled relatively precisely with PBPKs [17] and discrepancies between the model's prediction and experimental data obtained with the devices can point to gaps in our understanding (Fig. 1). In fact, when the devices are designed physiologically correct, it might even be possible to determine data for parameters in PBPK models from observations using the microdevices. The resulting models can be used to predict human response to a wide variety or combination of inputs with higher accuracy than before.

2.4. Individualized medicine

Development of the pharmacogenetic and pharmacogenomic sciences, which focus on the analysis of patient-specific responses to drugs based on variations in genotype, has since given rise to the concept of personalized medicine [72–74]. Similarly, the use of biopsy samples, stem cells, or induced pluripotent stem (iPS) cells, derived

from patient tissue, could be used in personalized assays, recapitulating patient-specific responses in vitro. Since multi-organ microdevices can be operated with small numbers of cells, they could potentially be used to model multiple relevant organ functions for the assessment of drug responses in the context of a patient's individual disease state [75,76]. Van Midwoud et al. have successfully demonstrated that it is possible to utilize intestinal and liver tissue slices from animals within a multi-organ microdevice and yield metabolic activity that was comparable to that obtained in conventional culture for several hours of device operation [9]. The interplay between the intestine and the liver was demonstrated by exposing the slices to the primary bile acid. Although this study did not utilize human tissue samples, the feasibility of incorporating tissue samples from patient biopsies was shown.

Depending on the patient, the use of stem cells or induced pluripotent stem cells could have a number of advantages over the use of primary cells and tissue samples, especially when developing drug formulations for the central nervous system (CNS) and treatment of degenerative diseases. Stem cells could facilitate experiments with cell types that remain difficult to obtain from biopsies, such as neurons of the CNS. Once obtained and developed, induced pluripotent stem cells can be expanded through multiple passages, thereby providing many more cells than originally taken from the patient. Since more cells are available from a single tissue sample, samples that need to be taken from the patient can be reduced in size. Reducing tissue damage is particularly important for patients with degenerative diseases such as muscular dystrophy. For these patients, taking samples from unaffected organs such as the skin could be considered as well. It should be noted however, that iPS cells take time to develop, so the need for the drug treatment must not be acute when attempting to utilize these cells for personalized assays.

3. Challenges

3.1. Device development

In order to benefit from experiments with multi-organ microdevices, these systems must reliably replicate human metabolism or at least a subset of human metabolism. While several multi-organ microdevices have been developed for the purpose of demonstrating their usefulness in the drug development process, there are practical challenges that must be overcome if the devices are to be used by the pharmaceutical industry. In regard to device development, current efforts to overcome these challenges aim at improving the usability of the devices and the authenticity with which the human metabolism is mimicked. Currently, in the US, the Defense Advanced Research Projects Agency (DARPA) and the National Institute of Health (NIH) are substantially funding research efforts (Microphysiological Systems Program) towards this goal [77]. In particular, the funded research focuses on developing systems that support the culture of primary cells and stem cells for an extended period of time. These efforts also include the development of a common blood surrogate (cell culture medium). Below we discuss these challenges in more detail.

3.1.1. Device design

Designing multi-organ microdevices in a physiologically relevant manner increases the predictive power of data obtained from their use. Designing organ compartments in a non-physiologic manner can lead to an overproduction or underproduction of relevant metabolites. For example, if the liver compartment is larger than it should be according to physiological scaling and has a cellular construct of biological activity similar to natural tissue, toxic metabolites that are generated in the liver compartment will reach other tissues at a higher concentration and cause proportionately more damage than would be the case if the liver was appropriately scaled. One approach to scaling that has been used is calculating on-chip organ chamber sizes according to the needed fluid residence time within each organ chamber [4–8]. In vivo, the blood residence time within organs depends on the size of the organ, the composition of the tissue, and the rate of perfusion. In the device the fluid flow rate, the organ chamber size, and the composition of the tissue within determine the fluid residence time within each organ chamber. The fluid flow rate within each organ is a percentage of the overall flow (or recirculation) rate and relates to the fraction of blood distribution to each organ in vivo. Since fluid residence times per organ and the percent of total blood that reaches each organ (i.e. percent of total flow) are given by in vivo values, the chamber volume can be calculated so that a given fluid residence time is achieved for a particular tissue model under a particular flow rate. The volume of free liquid to cells in the device should be similar to that in the body. Data that were obtained with devices that were scaled according to fluid residence times are most accurate if the chambers contain 3D tissue-like constructs and there is less than 200 μm of a distance between any cells and the medium supply. This close proximity of medium insures oxygen availability throughout the organ mimic. Although other design approaches are possible, these principles were considered when designing several devices that were discussed earlier [4–8]. If the tissue constructs used in the device have a different activity than the native tissues (for example in a disease model), other metabolic processes such as matching the degree of conversion of a major nutrient may be used as a design criterion.

Downscaling the organ chamber volumes as much as possible is an advantage if the cell sources are expensive. There must be, however, a minimum number of cells in the smallest organ chamber that is represented in the device. The smallest organ chamber should contain enough cells so that a meaningful metabolite concentration can be generated. From a practical point of view, handling very small numbers of cells (e.g. less than 100) might make an accurate device setup very difficult. Another consideration for establishing a minimum size of the device is the potential need to take medium from the device for subsequent metabolite analysis. The overall amount of medium within the device needs to be large enough so that a minimum of medium could be withdrawn without significantly perturbing the system (e.g. 25%). At the same time, some organs of the human body are very small (such as the pituitary glands) and would lead to a relatively large system if included in the device. Including such small organs implicitly in an “other organ” compartment rather than explicitly as an organ chamber that is populated with cells might be more practical for drug applications in which these organs do not play an important role.

More broadly, all tissues should be included either explicitly or implicitly if the devices are to be physiologic. For any organ or tissue that does not metabolize, absorb, or respond to the test compound or its metabolites, that organ/tissue can be included into an “other tissues” compartment. Such compartments do not have cells, but emulate the hold up of the fluid within that tissue (blood and interstitial fluid). These compartments may be divided into “rapidly perfused” and “poorly perfused” compartments. Such tissue compartments, even without cells, are included in PBPK models to capture the appropriate dynamics and must be included in multi-organ microdevices to mimic the appropriate distribution and dynamics of the body's response to a drug or chemical compound.

3.1.2. The development of a common cell culture medium

In vitro cell-cultures are designed to mimic the relevant in vivo environment. A temperature of 37 °C, and a controlled humidified gas mixture of 5% CO_2 and 95% O_2 are the standard physical conditions, while a blood surrogate medium with appropriate micro and macronutrients is used to recreate the chemical milieu. Cell culture media have evolved from a salt solution to preserve tissue, to more complex compositions able to maintain cells and tissues in an active state for extended periods of time. Different cell types often require the use of different cell culture media. That these different requirements can present a challenge for multi-organ microdevices was demonstrated with a device that showed tissue specific responses to a stimulant The device

combined tissues of the liver, lung, kidney, and adipose within one platform [8,78]. The tissues were stimulated with TGF-ß1, indicating a dose-dependent response. TGF-ß1 supported the growth of A549 lung cells, but inhibited the growth of HepG2/C3A liver cells. This response highlights the difficulty of finding a common medium with growth factors that support the viability of all cell types. This is a challenge for the development of multi-organ microdevices that must be solved in order for these devices to move forward. In this particular study the authors present an approach that uses gelatin microspheres to release TGF-ß1 locally to support the lung compartment while maintaining low levels of TGF-ß1in the circulation. This system demonstrated that the four tissues could remain viable within one device and that differential tissue response to TGF-ß1 could be emulated.

Human or animal sera, the most commonly used being fetal bovine serum, are often employed to supplement basic medium since they contain essential compounds for the growth and maintenance of cells and mimic many of the transport properties of blood. Recently, developing a serum-free medium has been a goal of many investigators. A serum-free formulation improves the consistency and definition of the culture medium. Since variation in the serum composition has been known to affect cell culture maintenance and subsequent experimental data, we can expect that serum-free formulations will improve the quality of experimental data [79]. Serum-free medium formulations are based on the addition of cell-specific growth factors and supplements to a common base medium, a strategy that facilitates the correct maintenance of specific cell cultures [80]. For example, the first serum-free defined culture system for hippocampal neurons was published by Hickman [81], and this model has since been adapted to facilitate the maintenance of cardiomyocytes [82], motoneurons [83], sensory neurons [84], and skeletal muscle cells [85] in defined in vitro conditions.

The recent development of novel multi-organ microdevices requires the development of advanced medium formulations, a challenge that arises from the need to preserve each cell type's morphology and function while in co-culture. This need was sufficiently addressed in the multi-organ studies discussed in Section 2, however, all media used for these studies were based on formulations that contain serum, Recent attempts to find a common medium formulation without serum have also been published: i) Davis and co-workers have observed that oligodendrocyte precursors can be differentiated into mature oligodendrocytes that express myelin basic protein, using a serum-free medium in co-culture with rat embryonic motoneurons on a non-biological substrate [86]. ii) Guo et al. used a commercialized medium containing Neurobasal, B27, creatine, estrogen and cholesterol to promote neuromuscular junction formation between human stem cell-derived motoneurons and human skeletal muscle [87]. Despite this progress, successful co-culture of more than four cell types in a common medium is difficult, and further investigations are needed to increase the number of cell types that can be maintained within a single platform.

3.1.3. Cell sources

Animal models are typically the primary source for most cell types utilized in experimental cultures. However, as already stated, their low predictive power, with regards to human responses to novel therapeutic treatment, makes them a poor candidate for use in microdevices that are to be used for drug development. Certain primary human cell types, such as skin [88], skeletal muscle [89], and blood [90], are relatively easy to obtain. Acquisition of others, such as neurons, is more problematic due to the trauma caused by extraction, and in such cases investigators are often limited to cadaver tissue as a cell source [91]. As a result, either embryonic or induced pluripotent stem cells have become an attractive alternative for investigators seeking to model human tissue function in vitro [91].

Stem cell technologies are attractive to investigators developing microdevices for drug development applications, since they facilitate the production of cell lines maintaining stable transfections [92]. Such genetic manipulation can be used to produce functional human cell types carrying a fluorescent reporter gene, conjugated to a specific promoter, to allow optical assessment of metabolic activity in response to therapeutic treatment [93]. Furthermore, the application of induced pluripotent stem cell technology from specific patients makes the concept of personalized medicine and patient specific disease models a possibility.

Widespread adoption of stem cells, particularly induced pluripotent stem cells, for in vitro applications has been questioned due to the reliability of the available cell lines. Such cell's ability to successfully differentiate into specific lineages has been found to vary based on differences in donor genotype and tissue of origin [94]. Moreover, since stem cells are incredibly susceptible to differentiation induction based on their physical and chemical micro-environments, different labs have occasionally produced conflicting data, or been unable to recreate the work of others, calling into question the validity of certain differentiation protocols [95,96]. Likewise, human embryonic stem cell lines, while sharing certain gene expression profiles, have been found to possess differences in the expression of several lineage markers [97]. Consequently, although numerous commercially available stem cell lineages are available, some investigators continue to focus on the use of primary tissue from human and animal models as a more consistent and reliable alternative. Although primary cells vary from donor to donor, they typically maintain full, differentiated function while stem cell based constructs may not display the full adult phenotype. Efforts are underway to generate comprehensive selection criteria and universal preparation standards for stem cell production [94], but what remains clear is that a more complete understanding of stem cell development and functional capacity is necessary to advance the development of next-generation human in vitro assays.

Such systems would be of tremendous benefit to the study of human genetic conditions for which no animal model exists or in instances when animal models fail to wholly recapitulate the complexity of the human condition [76]. For example, induced pluripotent stem cells have recently been used to model the electrophysiological profile of cardiomyocytes from a patient with type 1 long QT syndrome in vitro [98]. Since substantial differences in cardiac physiology exist between humans and rodents, the use of mice or rats is unsuitable for investigating this condition, and highlights the importance of novel in vitro platforms for developing new therapies. Similarly, studies have been performed using induced pluripotent stem cells derived from patients with familial Parkinson's disease [99], as well as familial and sporadic Alzheimer's disease [100], to investigate specific cellular responses and physiological differences in cells possessing these common aberrant genotypes.

As mentioned previously, given the variability in performance of stem cell lines, development of stringent selection criteria and culture parameters are necessary to facilitate the widespread adoption of such cells into high throughput assays and screening systems. Use of these cells in multi-organ microdevices is a goal yet to be realized on a routine basis. However, it remains an exciting prospect for improving patient care and the understanding of specific disease states, as well as their responses to novel therapeutics.

3.1.4. Authenticity of cellular behavior

Once an appropriate cell type is obtained, a further problem is the method of maintaining these cells within a housing that permits full functionality and correct emulation of in vivo behavior. Here single tissue models can be used to optimize tissue behavior. Three-dimensionality has been shown to create more authentic tissue responses than two-dimensional tissues [101]. Three-dimensional tissues and multi-cell type tissues that were cultured within microfluidic devices have been developed for the liver and other tissues [26,29,30,32,33,40,66]. While traditional in vitro assays often focus on measurement of biomarkers as indicators of cell health and

functionality, direct measurement of functional output is a more accurate method, and likely to yield data with stronger correlative power to clinical observations. To that end, a number of groups have recently focused on the development of "on-chip" in vitro assay systems capable of emulating selected functional responses of key organs and tissues. Examples of such technologies can be found for heart [40,41], liver [18–29,102], lung [37–39], kidney [46], skeletal muscle [87,103], hippocampus [104], gastro-intestinal tract [8,31–33,102,105], and skin [35,36] among others.

Assessing authentic tissue behavior in multi-organ micro-devices requires the real-time recording of primary functional outputs of different cell types. Physical movement of contractile cell types, such as skeletal muscle myotubes, cardiomyocytes and smooth muscle cells can be evaluated through measurement of substrate deflection, either by use of cantilevers [103] or flexible posts [106]. Electrical activity of neurons is usually assessed in vitro using electrophysiological patch clamp recordings [107], however, such techniques are invasive and difficult to scale up for high throughput applications. Use of microelectrode arrays facilitate the high throughput interrogation of cultured neuronal networks in a high throughput manner, and are far more amenable to integration with multi-organ platforms [104]. Moreover, the use of microelectrodes can also be used as a means to assess the concentrations of a wide variety of functionally relevant analytes such as superoxide radicals [108] and lactate [109]. Optical techniques can also be used to assess functional metabolism of drug compounds and their effect on cell viability in microdevices [4–8,23].

Without the means to measure and assess the biomimicity of cultured tissue analogs, very little of the data required for accurate predictions of in vivo drug responses can be obtained. Development of appropriate analytical techniques for application within novel multi-organ micro-devices is essential as a means to assess the appropriate real time physiological and functional effects of drug treatment in vitro.

3.2. Commercialization

Commercial development of multi-organ microdevices for drug testing is currently underway. For example, Hurel corporation discusses a microfluidic platform for testing two fluidically interconnected chambers with cells/tissues, a medium reservoir and in situ "pumps" for moving fluid. This device is currently in beta testing and will presumably be commercially available in the near future.

In order for the pharmaceutical industry to adopt new devices, they must be easy to use and provide a profit by being lower in cost than conventional approaches. Low cost of the physical devices can be achieved by utilizing polymeric materials for device fabrication and device designs that are pumpless and valveless, or have low cost strategies for moving fluids in a controllable manner. However, there are other challenges that must be overcome in order to fulfill the ease of use and low cost requirement. Some of these challenges are discussed below.

3.2.1. Longevity

The need to maintain in vitro models for extended periods of time is of great importance for drug development applications that aim to predict the effects of chronic drug exposure. Prolonged metabolite exposure and waste build-up within the organ compartments limit the lifetime of multi-organ microdevices. In addition, long in vitro culture periods tend to lead to cellular senescence or induction of apoptotic pathways, which can confound data from toxicity studies. Furthermore, typically cell maturation takes a few hours up to two or three weeks in culture. This requires coordinating the cell seeding process so that all cells are mature and functional at the time of the experiment. Complex culture environments are often necessary to produce accurate models of in vivo tissues. However, the incorporation of increasing numbers of disparate cell types into common culture conditions makes the maintenance of such platforms problematic since the conservation of optimized parameters for all cell types becomes more and more difficult. One solution to this problem may be to culture each cell type in its separate medium until the cells reach maturation and operate the device with a common medium for the duration of the experiment. Using this procedure, devices have been operated for 24–72 h without any media exchange [5].

Relatively long culture periods have been established for certain individual cell types. For example, skeletal muscle cultures have been shown to survive in vitro for up to 90 days, during which time they promote phenotype maturation, as evidenced by quantifiable changes in myosin heavy chain isoform composition [110]. This system was subsequently modified to support the long-term (30+ days) co-culture and functional interaction of skeletal muscle myotubes and motoneurons [111]. Such data demonstrate that the maintenance of co-cultures is possible over long periods provided that careful consideration is given to culture variables such as surface chemistry and topography, medium formulation and correct temporal addition of exogenous stimuli. The described data were obtained using rodent cells, however, similar studies using human cells were successful in co-cultures for 10 days [87]. Such disparity is likely due to inherent differences in rodent and human cellular maturation and maintenance, and highlights the need for further assessment of optimal culture conditions to promote more long-term survival for human cells, especially stem cells. It should be noted that data pertaining to the long-term survival and functional viability of human neuronal cell types has been reported [107,112], so the possibility of functional nerve-muscle co-cultures for extended periods of time using human cells seems possible.

3.2.2. Validation and standardization

The exponential development of microfabricated devices has led to a variety of available tools dedicated to multiple cell cultures within one device. However, the approaches all differ by materials, configuration, and criteria of design. All of these variables have the potential to influence cellular behavior in multi-organ microdevices. Proof of concept experiments with the described multi-organ devices have provided some evidence for their usefulness in early stage drug testing. However, if the devices are to be used in industry there is a real need for biological validation and standardization. The currently funded efforts by DARPA and NIH aim specifically to develop standard platforms that can be used by many different groups to utilize multi-organ microsystems. The development of such biological platform requires finding a balance between complexity, required by the need to recreate the in vivo situation accurately, and the need for the devices to be inexpensive and easy to use. The approach used by the cosmetic industry to replace animal testing, as mandated by ECVAM in Europe and encouraged by the ICCVAM program in the US, could provide some direction for the development of such standards. A mutual endpoint toolbox could allow inter-laboratory and inter-platform evaluation. Quantitatively and qualitatively assessment of a platform's functionality would increase our confidence in the results obtained with this system. In this regard, a multi-organ platform dedicated to drug development should focus on the ADMET process with relevant biomarkers of early toxicity for each organ. For example, a common method to evaluate the potential of a drug to induce CYP450 enzyme expression, which can be an early response indicating drug toxicity, has been proposed by ECVAM [113]. A cocktail of specific probes were designed as a calibrator for the four main CYP enzymes involved in drug metabolism. Using the same cocktail of drugs and evaluation methods across laboratories will allow the validation of different models in a defined way. Working towards standardization will require a close collaboration between engineers, biologists, and the pharmaceutical industry.

4. Conclusions

Multi-organ microdevices have the potential to contribute to the early stage drug development process in ways that are not possible

with conventional in vitro models. Because the devices are designed to mimic the physiologic relationship of organs and their interaction via soluble metabolites, they can capture inter-organ effects in vitro. The low cost of the devices permits testing a large number of drugs and drug combinations with human tissues instead of animal tissues. This bears the potential advantage of providing a higher degree of accuracy when predicting toxic side effects for humans. Further, the devices can easily be modified to mimic disease conditions or entirely un-physiologic conditions that allow for an increase in flexibility when assessing with new drug candidates. Here we have reviewed and discussed studies that have demonstrated the devices' capability to simulate the first pass metabolism, the conversion of anti-cancer prodrugs and their subsequent effects on tumor tissue, the synergistic actions of two MDR modulators, and the modulation of bioavailability and toxicity via barrier tissues and tissues that absorb and store chemical compounds. Most importantly, we have discussed how the devices can be used to test hypotheses concerning mechanistic models of drug toxicity. Experimenting with multi-organ microdevices gives us the ability to obtain useful, and often non-obvious information, on biological mechanism and the exchange of metabolites between tissues.

However, significant challenges must be overcome in order for the devices to become relevant for the pharmaceutical industry. Currently, DARPA and NIH are funding efforts in the US that will likely result in several platforms that can be used by investigators. The efforts also have the potential to solve some of the most important questions regarding the authenticity of the mimicked metabolism since all developments are required to utilize primary or stem cell sources. In addition, the resulting collaborations are a strong catalyst for the development and combining of ideas. In general, we can expect to see an increase of the total number of organs that are explicitly included in multi-organ microdevices.

Disclosure

MLS is on the Hurel scientific advisory board.

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References

[1] J.A. Dimasi, L. Feldman, A. Seckler, A. Wilson, Trends in risks associated with new drug development: success rates for investigational drugs, Clin. Pharmacol. Ther. 87 (3) (2010) 272.
[2] J.A. Dimasi, H. Grabowski, The cost of biopharmaceutical R&D: is biotech different? Manag. Decis. Econ. 28 (469) (2007) 469–479.
[3] R. Greek, A. Menache, Systematic reviews of animal models: methodology versus epistemology, Int. J. Med. Sci. 10 (3) (2013) 206–221. http://dx.doi.org/10.7150/ijms.5529.
[4] D.A. Tatosian, M.L. Shuler, A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers, Biotechnol. Bioeng. 103 (1) (2009) 187–198. http://dx.doi.org/10.1002/bit.22219.
[5] J.H. Sung, M.L. Shuler, 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 (10) (2009) 1385–1394. http://dx.doi.org/10.1039/b901377f.
[6] K. Viravaidya, A. Sin, M.L. Shuler, Development of a microscale cell culture analog to probe naphthalene toxicity, Biotechnol. Prog. 20 (1) (2004) 316–323. http://dx.doi.org/10.1021/bp0341996.
[7] K. Viravaidya, M.L. Shuler, Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies, Biotechnol. Prog. 20 (2) (2004) 590–597. http://dx.doi.org/10.1021/bp034238d.
[8] G.J. Mahler, M.B. Esch, R.P. Glahn, M.L. Shuler, Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity, Biotechnol. Bioeng. 104 (1) (2009) 193–205. http://dx.doi.org/10.1002/bit.22366.
[9] P.M. van Midwoud, M.T. Merema, E. Verpoorte, G.M.M. Groothuis, A microfluidic approach for in vitro assessment of interorgan interactions in drug metabolism using intestinal and liver slices. Lab on a Chip, R. Soc. Chem. 10 (20) (2010) 2778.
[10] I. Wagner, E.-M. Materne, S. Brincker, U. Süßbier, C. Frädrich, M. Busek, et al., A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture, Lab on a Chip. 2013 ed. The Royal Society of Chemistry, 13(18), 2013, p. 3538.
[11] R. Brand, T. Hannah, C. Mueller, Y. Cetin, A novel system to study the impact of epithelial barriers on cellular metabolism, Ann. Biomed. Eng. 28 (2000) 1210–1217.
[12] M.B. Esch, T.L. King, M.L. Shuler, The role of body-on-a-chip devices in drug and toxicity studies, Annu. Rev. Biomed. Eng. 13 (2011) 55–72.
[13] M. Baker, A living system on a chip, Nature 471 (7340) (2011) 661–665.
[14] A.P. Li, The use of the integrated discrete multiple organ co-culture (IdMOC (R)) system for the evaluation of multiple organ toxicity, ATLA Altern. Lab. Anim. 37 (4) (2009) 377.
[15] B. Davies, T. Morris, Physiological parameters in laboratory animals and humans, Pharm. Res. 10 (7) (1993) 1093–1095.
[16] P.S. Price, R.B. Conolly, C.F. Chaisson, E.A. Gross, J.S. Young, E.T. Mathis, et al., Modeling interindividual variation in physiological factors used in PBPK models of humans, Critical Reviews in Toxicology, 33(5), Informa UK Ltd., UK, 2003. 469.
[17] J.H. Sung, M.B. Esch, M.L. Shuler, Integration of in silico and in vitro platforms for pharmacokinetic-pharmacodynamic modeling, Expert Opin. Drug Metab. Toxicol. 6 (9) (2010) 1063–1081.
[18] E. Novik, T.J. Maguire, P. Chao, K.-C. Cheng, M.L. Yarmush, A microfluidic hepatic coculture platform for cell-based drug metabolism studies, Biochem. Pharmacol. 79 (7) (2010) 1036–1044.
[19] S. Kidambi, R.S. Yarmush, E. Novik, P. Chao, M.L. Yarmush, Y. Nahmias, Oxygen-mediated enhancement of primary hepatocyte metabolism, functional polarization, gene expression, and drug clearance, Proc. Natl. Acad. Sci. 106 (37) (2009) 15714–15719. http://dx.doi.org/10.1073/iti0937106.
[20] P. Chao, T. Maguire, E. Novik, K. Cheng, Evaluation of a microfluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human, Biochem. Pharmacol. 78 (6) (2009) 625–632.
[21] S.-A. Lee, E. Kang, J. Ju, D.-S. Kim, S. Lee, Spheroid-based three-dimensional liver-on-a-chip to investigate hepatocyte–hepatic stellate cell interactions and flow effects, Lab Chip 13 (18) (2013) 3435–3766.
[22] J. Schütte, B. Hagmeyer, F. Holzner, M. Kubon, S. Werner, C. Freudigmann, et al., "Artificial micro organs"—a microfluidic device for dielectrophoretic assembly of liver sinusoids, Biomed. Microdevices 13 (3) (2011) 493–501. http://dx.doi.org/10.1007/s10544-011-9517-7.
[23] B. Ma, G. Zhang, J. Qin, B. Lin, Characterization of drug metabolites and cytotoxicity assay simultaneously using an integrated microfluidic device, Lab Chip 9 (2) (2009) 232–238. http://dx.doi.org/10.1039/b809117j.
[24] C. Zhang, S.-M. Chia, S.-M. Ong, S. Zhang, Y.-C. Toh, D. van Noort, H. YU, The controlled presentation of TGF-β1 to hepatocytes in a 3D-microfluidic cell culture system, Biomaterials 30 (23) (2009) 3847–3853.
[25] S.M. Hattersley, J. Greenman, S.J. Haswell, Study of ethanol induced toxicity in liver explants using microfluidic devices, Biomed. Microdevices 13 (6) (2011) 1005–1014. http://dx.doi.org/10.1007/s10544-011-9570-2.
[26] M.J. Powers, K. Domansky, M.R. Kaazempur-Mofrad, A. Kalezi, A. Capitano, A. Upadhyaya, et al., A microfabricated array bioreactor for perfused 3D liver culture, Biotechnol. Bioeng. 78 (3) (2002) 257–269. http://dx.doi.org/10.1002/bit.10143.
[27] M.J. Powers, D.M. Janigian, K.E. Wack, C.S. Baker, D.B. Stolz, L.G. Griffith, Functional behavior of primary rat liver cells in a three-dimensional perfused microarray bioreactor, Tissue Eng. 8 (3) (2002) 499–513. http://dx.doi.org/10.1089/107632702760184745.
[28] L.G. Griffith, B. WU, M.J. CIMA, M.J. Powers, B. CHAIGNAUD, J.P. Vacanti, In vitro organogenesis of liver tissue, Ann. N. Y. Acad. Sci. 831 (1) (2006) 382–397. http://dx.doi.org/10.1111/j.1749-6632.1997.tb52212.x.
[29] K. Domansky, W. Inman, J. Serdy, A. Dash, M.H.M. Lim, L.G. Griffith, Perfused multiwell plate for 3D liver tissue engineering, Lab Chip 10 (1) (2010) 51–58. http://dx.doi.org/10.1039/B913221J.
[30] R. Kostadinova, F. Boess, D. Applegate, L. Suter, T. Weiser, T. Singer, et al., A long-term three dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity, Toxicol. Appl. Pharmacol. 268 (1) (2013) 1–16. http://dx.doi.org/10.1016/j.taap.2013.01.012.
[31] Y. Imura, Y. Asano, K. Sato, E. Yoshimura, A microfluidic system to evaluate intestinal absorption, Anal. Sci. 25 (12) (2009) 1403–1407.
[32] J.H. Sung, Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model, Lab Chip 11 (3) (2011) 389–392.
[33] J. Pusch, M. Votteler, S. Göhler, J. Engl, M. Hampel, H. Walles, K. Schenke-Layland, The physiological performance of a three-dimensional model that mimics the microenvironment of the small intestine, Biomaterials 32 (30) (2011) 7469–7478. http://dx.doi.org/10.1016/j.biomaterials.2011.06.035.
[34] M.B. Esch, J.H. Sung, J. Yang, C. Yu, J. Yu, J.C. March, M.L. Shuler, On chip porous polymer membranes for integration of gastrointestinal tract epithelium with

microfluidic "body-on-a-chip" devices, Biomed. Microdevices 14 (5) (2012) 895–906. http://dx.doi.org/10.1007/s10544-012-9669-0.
[35] E. Brauchle, H. Johannsen, S. Nolan, S. Thude, K. Schenke-Layland, Design and analysis of a squamous cell carcinoma in vitro model system, Biomaterials 34 (30) (2013) 7401–7407. http://dx.doi.org/10.1016/j.biomaterials.2013.06.016.
[36] B. Ataç, I. Wagner, R. Horland, R. Lauster, U. Marx, A.G. Tonevitsky, et al., Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion, Lab Chip 13 (18) (2013) 3555–3561. http://dx.doi.org/10.1039/C3LC50227A.
[37] S. Hinderer, M. Schesny, A. Bayrak, B. Ibold, M. Hampel, T. Walles, et al., Engineering of fibrillar decorin matrices for a tissue-engineered trachea, Biomaterials 33 (21) (2012) 5259–5266. http://dx.doi.org/10.1016/j.biomaterials.2012.03.075.
[38] D. Huh, B. Matthews, A. Mammoto, M. Montoya-Zavala, Reconstituting organ-level lung functions on a chip, Science (2010) 1662–1668.
[39] D. Huh, H. Fujioka, Y.-C. Tung, N. Futai, R. Paine, J.B. Grotberg, et al., Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems, Proc. Natl. Acad. Sci. 104 (48) (2007) 18886.
[40] M.B. Chen, S. Srigunapalan, A.R. Wheeler, C.A. Simmons, A 3D microfluidic platform incorporating methacrylated gelatin hydrogels to study physiological cardiovascular cell–cell interactions, Lab Chip 13 (13) (2013) 2591–2598. http://dx.doi.org/10.1039/c3lc00051f.
[41] A. Agarwal, J.A. Goss, A. Cho, M.L. McCain, K.K. Parker, Microfluidic heart on a chip for higher throughput pharmacological studies, Lab Chip (2013). http://dx.doi.org/10.1039/c3lc50350j.
[42] M.B. Esch, D.J. Post, M.L. Shuler, T. Stokol, Characterization of in vitro endothelial linings grown within microfluidic channels, Tissue Eng. A 17 (23–24) (2011) 2965–2971.
[43] K. Wong, J.M. Chan, R.D. Kamm, Microfluidic models of vascular functions, Annu. Rev. Biomed. Eng. 14 (1) (2012) 205–230.
[44] K. Schimek, M. Busek, S. Brincker, B. Groth, S. Hoffmann, R. Lauster, et al., Integrating biological vasculature into a multi-organ-chip microsystem, Lab Chip 13 (18) (2013) 3588. http://dx.doi.org/10.1039/c3lc50217a.
[45] Y. Zheng, J. Chen, M. Craven, N.W. Choi, S. Totorica, A. Diaz-Santana, et al., In vitro microvessels for the study of angiogenesis and thrombosis, Proc. Natl. Acad. Sci. 109 (24) (2012) 9342–9347.
[46] B. Subramanian, D. Rudym, C. Cannizzaro, R. Perrone, J. Zhou, D.L. Kaplan, Tissue-engineered three-dimensional in vitro models for normal and diseased kidney, Tissue Eng. A 16 (9) (2010) 2821–2831. http://dx.doi.org/10.1089/ten.TEA.2009.0595.
[47] N. Ferrell, K.B. Ricci, J. Groszek, J.T. Marmerstein, W.H. Fissell, Albumin handling by renal tubular epithelial cells in a microfluidic bioreactor, Biotechnol. Bioeng. 109 (3) (2011) 797–803. http://dx.doi.org/10.1002/bit.24339.
[48] K.-J. Jang, K.-Y. Suh, A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells, Lab Chip 10 (1) (2010) 36–42. http://dx.doi.org/10.1039/B907515A.
[49] N. Ferrell, R.R. Desai, A.J. Fleischman, S. Roy, H.D. Humes, W.H. Fissell, A microfluidic bioreactor with integrated transepithelial electrical resistance (TEER) measurement electrodes for evaluation of renal epithelial cells, Biotechnol. Bioeng. 107 (4) (2010) 707–716.
[50] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends Cell Biol. 21 (12) (2011) 745–754. http://dx.doi.org/10.1016/j.tcb.2011.09.005.
[51] L. Shintu, R. Baudoin, V. Navratil, J.-M. Prot, C. Pontoizeau, M. Defernez, et al., Metabolomics-on-a-chip and predictive systems toxicology in microfluidic bioartificial organs, Anal. Chem. 84 (4) (2012) 1840–1848. http://dx.doi.org/10.1021/ac2011075.
[52] A.M. Ghaemmaghami, M.J. Hancock, H. Harrington, H. Kaji, A. Khademhosseini, Biomimetic tissues on a chip for drug discovery, Drug Discov. Today 17 (3–4) (2012) 173–181. http://dx.doi.org/10.1016/j.drudis.2011.10.029.
[53] P. Neuži, S. Giselbrecht, K. Länge, T.J. Huang, A. Manz, Revisiting lab-on-a-chip technology for drug discovery, Nat. Rev. Drug Discov. 11 (8) (2012) 620–632. http://dx.doi.org/10.1038/nrd3799.
[54] J.H. Sung, M.B. Esch, J.M. Prot, C.J. Long, A. Smith, J.J. Hickman, M.L. Shuler, Microfabricated mammalian organ systems and their integration into models of whole animals and humans, Lab Chip 13 (7) (2013) 1201–1212. http://dx.doi.org/10.1039/c3lc41017j.
[55] L. Choucha-Snouber, C. Aninat, L. Grsicom, G. Madalinski, C. Brochot, P.E. Poleni, et al., Investigation of ifosfamide nephrotoxicity induced in a liver–kidney co-culture biochip, Biotechnol. Bioeng. 110 (2) (2012) 597–608. http://dx.doi.org/10.1002/bit.24707.
[56] M. Malet-Martino, Clinical studies of three oral prodrugs of 5-fluorouracil (Capecitabine, UFT, S-1): a review, Oncologist 7 (4) (2002) 288–323. http://dx.doi.org/10.1634/theoncologist.7-4-288.
[57] T. Komatsu, H. Yamazaki, N. Shimada, M. Nakajima, T. Yokoi, Roles of cytochromes P450 1A2, 2A6, and 2C8 in 5-fluorouracil formation from tegafur, an anticancer prodrug, in human liver microsomes, Drug Metab. Dispos. 28 (12) (2000) 1457–1463.
[58] Y. Lau, Y. Chen, T. Liu, C. Li, X. Cui, Evaluation of a novel in vitro Caco-2 hepatocyte hybrid system for predicting in vivo oral bioavailability, Drug Metab. Dispos. 32 (9) (2004) 937–942.
[59] S. Hyung Choi, M. Nishikawa, A. Sakoda, Y. Sakai, 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 (3) (2004) 393–402.
[60] R. Brand, T. Hannah, C. Mueller, Y. Cetin, A novel system to study the impact of epithelial barriers on cellular metabolism, Ann. Biomed. Eng. (28) (2000) 1210–1217.
[61] S.H. Choi, O. Fukuda, A. Sakoda, Y. Sakai, 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 (3) (2004) 333–339.
[62] J.S. Gujral, T.R. Knight, A. Farhood, M.L. Bajt, H. Jaeschke, Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? Toxicol. Sci. 67 (2) (2002) 322–328.
[63] M.A. Guzzardi, F. Vozzi, A.D. Ahluwalia, Study of the crosstalk between hepatocytes and endothelial cells using a novel multicompartmental bioreactor: a comparison between connected cultures and cocultures, Tissue Engineering Part A 15 (11) (2009) 3635–3644.
[64] F. Vozzi, J.-M. Heinrich, A. Bader, A.D. Ahluwalia, Connected culture of murine hepatocytes and human umbilical vein endothelial cells in a multicompartmental bioreactor, Tissue Engineering Part A 15 (6) (2008) 1291–1299.
[65] P.J. Lee, P.J. Hung, L.P. Lee, An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture, Biotechnol. Bioeng. 97 (5) (2007) 1340–1346. http://dx.doi.org/10.1002/bit.21360.
[66] K.-W. Chang, C.-T. Lee, P. Tushar Harishchandra, H.-P. Chen, T.-R. Yueh, S. Valagerahally Puttaswamy, et al., 3D biomimetic chip integrated with microvascular system for studying the liver specific functions, Presented at the 2012 7th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS), IEEE, 2012, pp. 218–221. http://dx.doi.org/10.1109/NEMS.2012.6196760.
[67] E. Borowski, M.M. Bontemps-Gracz, A. Piwkowska, Strategies for overcoming ABC-transporters-mediated multidrug resistance (MDR) of tumor cells, Acta Biochim. Pol. 52 (3) (2005) 609–627.
[68] C. Pascaud, M. Garrigos, S. Orlowski, Multidrug resistance transporter P-glycoprotein has distinct but interacting binding sites for cytotoxic drugs and reversing agents, Biochem. J. 333 (1998) 351–358.
[69] M. Lehnert, W.S. Dalton, D. Roe, S. Emerson, S.E. Salmon, Synergistic inhibition by verapamil and quinine of P-glycoprotein-mediated multidrug resistance in a human myeloma cell line model, Blood 77 (2) (1991) 348–354.
[70] L.M. Sweeney, M.L. Shuler, J.G. Babish, A. Ghanem, A cell culture analogue of rodent physiology: application to naphthalene toxicology, Toxicol. in Vitro 9 (3) (1995) 307–316. http://dx.doi.org/10.1016/0887-2333(95)00007-U.
[71] A. Ghanem, M.L. Shuler, Combining cell culture analogue reactor designs and PBPK models to probe mechanisms of naphthalene toxicity, Biotechnol. Prog. 16 (3) (2000) 334–345. http://dx.doi.org/10.1021/bp9901522.
[72] R. Dienstmann, J. Rodon, J. Tabernero, Biomarker-driven patient selection for early clinical trials, Curr. Opin. Oncol. 25 (3) (2013) 305–312. http://dx.doi.org/10.1097/CCO.0b013e32835ff3cb.
[73] W. Kalow, Pharmacogenetics and pharmacogenomics: origin, status, and the hope for personalized medicine, Pharmacogenomics J. 6 (3) (2006) 162–165. http://dx.doi.org/10.1038/sj.tpj.6500361.
[74] M.J. Duffy, N. O'Donovan, J. Crown, Use of molecular markers for predicting therapy response in cancer patients, Cancer Treat. Rev. 37 (2) (2011) 151–159. http://dx.doi.org/10.1016/j.ctrv.2010.07.004.
[75] L.L. Rubin, K.M. Haston, Stem cell biology and drug discovery, BMC Biol. 9 (2011) 42. http://dx.doi.org/10.1186/1741-7007-9-42.
[76] G. Romano, F. Morales, I.R. Marino, A. Giordano, A commentary on iPS cell biology: potential applications in autologous tissue and cell transplantation, development of new models for the study of human pathological conditions and drug screening, J. Cell. Physiol. (2013). http://dx.doi.org/10.1002/jcp.24437.
[77] M.L. Sutherland, K.M. Fabre, D.A. Tagle, The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline, Stem Cell Res. Ther. 4 (Suppl. 1) (2013) I1.
[78] C. Zhang, Z. Zhao, N.A. Abdul Rahim, D. van Noort, H. Yu, Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments, Lab Chip 9 (22) (2009) 3185–3192. http://dx.doi.org/10.1039/b915147h.
[79] J. van der Valk, D. Brunner, K. De Smet, A. Fex Svenningsen, P. Honegger, L.E. Knudsen, et al., Optimization of chemically defined cell culture media—replacing fetal bovine serum in mammalian in vitro methods, Toxicol. in Vitro 24 (4) (2010) 1053–1063. http://dx.doi.org/10.1016/j.tiv.2010.03.016.
[80] D. Edwards, M. Das, P. Molnar, J.J. Hickman, Addition of glutamate to serum-free culture promotes recovery of electrical activity in adult hippocampal neurons in vitro, J. Neurosci. Methods 190 (2) (2010) 155–163. http://dx.doi.org/10.1016/j.jneumeth.2010.04.030.
[81] A.E. Schaffner, J.L. Barker, D.A. Stenger, J.J. Hickman, Investigation of the factors necessary for growth of hippocampal neurons in a defined system, J. Neurosci. Methods 62 (1–2) (1995) 111–119.
[82] A. Natarajan, M. Stancescu, V. Dhir, C. Armstrong, F. Sommerhage, J.J. Hickman, P. Molnar, Patterned cardiomyocytes on microelectrode arrays as a functional, high information content drug screening platform, Biomaterials 32 (18) (2011) 4267–4274. http://dx.doi.org/10.1016/j.biomaterials.2010.12.022.
[83] M. Das, P. Molnar, H. Devaraj, M. Poeta, J.J. Hickman, Electrophysiological and morphological characterization of rat embryonic motoneurons in a defined system, Biotechnol. Prog. 19 (6) (2003) 1756–1761. http://dx.doi.org/10.1021/bp034076l.
[84] J.W. Rumsey, M. Das, A. Bhalkikar, M. Stancescu, J.J. Hickman, Tissue engineering the mechanosensory circuit of the stretch reflex arc: sensory neuron innervation of intrafusal muscle fibers, Biomaterials 31 (32) (2010) 8218–8227. http://dx.doi.org/10.1016/j.biomaterials.2010.07.027.
[85] M. Das, C.A. Gregory, P. Molnar, L.M. Riedel, K. Wilson, J.J. Hickman, A defined system to allow skeletal muscle differentiation and subsequent integration with silicon microstructures, Biomaterials 27 (24) (2006) 4374–4380. http://dx.doi.org/10.1016/j.biomaterials.2006.03.046.

[86] H. Davis, M. Gonzalez, N. Bhargava, M. Stancescu, J.J. Hickman, S. Lambert, Rat cortical oligodendrocyte-embryonic motoneuron co-culture: an axon–oligodendrocyte interaction model, J. Biomater. Tissue Eng. 2 (3) (2012) 206–214.
[87] X. Guo, M. Gonzalez, M. Stancescu, H.H. Vandenburgh, J.J. Hickman, Neuromuscular junction formation between human stem cell-derived motoneurons and human skeletal muscle in a defined system, Biomaterials 32 (36) (2011) 9602–9611. http://dx.doi.org/10.1016/j.biomaterials.2011.09.014.
[88] Y. Sakai, M. Koike, H. Hasegawa, K. Yamanouchi, A. Soyama, M. Takatsuki, et al., Rapid fabricating technique for multi-layered human hepatic cell sheets by forceful contraction of the fibroblast monolayer, PLoS One 8 (7) (2013) e70970. http://dx.doi.org/10.1371/journal.pone.0070970.
[89] P. Scherp, N. Putluri, G.J. LeBlanc, Z.Q. Wang, X.H. Zhang, Y. Yu, et al., Proteomic analysis reveals cellular pathways regulating carbohydrate metabolism that are modulated in primary human skeletal muscle culture due to treatment with bioactives from *Artemisia dracunculus* L, J. Proteomics 75 (11) (2012) 3199–3210. http://dx.doi.org/10.1016/j.jprot.2012.03.024.
[90] A. Welin, F. Amirbeagi, K. Christenson, L. Bjorkman, H. Bjornsdottir, H. Forsman, et al., The human neutrophil subsets defined by the presence or absence of OLFM4 both transmigrate into tissue in vivo and give rise to distinct NETs in vitro, PLoS One 8 (7) (2013) e69575. http://dx.doi.org/10.1371/journal.pone.0069575.
[91] E.S. Fakunle, J.F. Loring, Ethnically diverse pluripotent stem cells for drug development, Trends Mol. Med. 18 (12) (2012) 709–716. http://dx.doi.org/10.1016/j.molmed.2012.10.007.
[92] L. Gerrard, D. Zhao, A.J. Clark, W. Cui, Stably transfected human embryonic stem cell clones express OCT4-specific green fluorescent protein and maintain self-renewal and pluripotency, Stem Cells 23 (1) (2005) 124–133. http://dx.doi.org/10.1634/stemcells.2004-0102.
[93] Development of a stable dual cell-line GFP expression system to study estrogenic endocrine disruptors, Available from: http://onlinelibrary.wiley.com/doi/10.1002/bit.21991/abstract2008.
[94] W. Wagner, A.D. Ho, Mesenchymal stem cell preparations—comparing apples and oranges, Stem Cell Rev. 3 (4) (2007) 239–248. http://dx.doi.org/10.1007/s12015-007-9001-1.
[95] C.M. Morshead, P. Benveniste, N.N. Iscove, D. van der Kooy, Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations, Nat. Med. 8 (3) (2002) 268–273. http://dx.doi.org/10.1038/nm0302-268.
[96] R. Raedt, J. Pinxteren, A. Van Dycke, A. Waeytens, D. Craeye, F. Timmermans, et al., Differentiation assays of bone marrow-derived Multipotent Adult Progenitor Cell (MAPC)-like cells towards neural cells cannot depend on morphology and a limited set of neural markers, Exp. Neurol. 203 (2) (2007) 542–554. http://dx.doi.org/10.1016/j.expneurol.2006.09.016.
[97] O. Adewumi, B. Aflatoonian, L. Ahrlund-Richter, M. Amit, P.W. Andrews, G. Beighton, et al., Characterization of human embryonic stem cell lines by the International Stem Cell Initiative, Nat. Biotechnol. 25 (7) (2007) 803–816. http://dx.doi.org/10.1038/nbt1318.
[98] I. Itzhaki, L. Maizels, I. Huber, L. Zwi-Dantsis, O. Caspi, A. Winterstern, et al., Modelling the long QT syndrome with induced pluripotent stem cells, Nature 471 (7337) (2011) 225–229. http://dx.doi.org/10.1038/nature09747.
[99] M.J. Devine, M. Ryten, P. Vodicka, A.J. Thomson, T. Burdon, H. Houlden, et al., Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus, Nat. Commun. 2 (2011) 440. http://dx.doi.org/10.1038/ncomms1453.
[100] M.A. Israel, S.H. Yuan, C. Bardy, S.M. Reyna, Y. Mu, C. Herrera, et al., Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells, Nature 482 (7384) (2012) 216–220. http://dx.doi.org/10.1038/nature10821.
[101] V.M. Weaver, O.W. Petersen, F. Wang, C.A. Larabell, P. Briand, C. Damsky, M.J. Bissell, Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies, J. Cell Biol. 137 (1) (1997) 231–245.
[102] F. Antunes, F. Andrade, F. Araújo, D. Ferreira, B. Sarmento, Establishment of a triple co-culture in vitro cell models to study intestinal absorption of peptide drugs, Eur. J. Pharm. Biopharm. 83 (2013) 427-435. http://dx.doi.org/10.1016/j.ejpb.2012.10.003.
[103] K. Wilson, M. Das, K.J. Wahl, R.J. Colton, J. Hickman, Measurement of contractile stress generated by cultured rat muscle on silicon cantilevers for toxin detection and muscle performance enhancement, PLoS One 5 (6) (2010). http://dx.doi.org/10.1371/journal.pone.0011042.
[104] A. Natarajan, T.B. DeMarse, P. Molnar, J.J. Hickman, Engineered in vitro feed-forward networks, J. Biotechnol. Biomater. 3 (153) (2013) 2.
[105] G.J. Mahler, M.L. Shuler, R.P. Glahn, 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 (7) (2009) 494–502.
[106] H. Vandenburgh, J. Shansky, F. Benesch-Lee, V. Barbata, J. Reid, L. Thorrez, et al., Drug-screening platform based on the contractility of tissue-engineered muscle, Muscle Nerve 37 (4) (2008) 438–447. http://dx.doi.org/10.1002/mus.20931.
[107] X. Guo, K. Johe, P. Molnar, H. Davis, J. Hickman, Characterization of a human fetal spinal cord stem cell line, NSI-566RSC, and its induction to functional motoneurons, J. Tissue Eng. Regen. Med. 4 (3) (2010) 181–193. http://dx.doi.org/10.1002/term.223.
[108] X.J. Chen, A.C. West, D.M. Cropek, S. Banta, Detection of the superoxide radical anion using various alkanethiol monolayers and immobilized cytochrome c, Anal. Chem. 80 (24) (2008) 9622–9629. http://dx.doi.org/10.1021/ac800796b.
[109] I.A. Ges, F. Baudenbacher, Enzyme-coated microelectrodes to monitor lactate production in a nanoliter microfluidic cell culture device, Biosens. Bioelectron. 26 (2) (2010) 828–833. http://dx.doi.org/10.1016/j.bios.2010.05.030.
[110] M. Das, J.W. Rumsey, N. Bhargava, M. Stancescu, J.J. Hickman, Skeletal muscle tissue engineering: A maturation model promoting long-term survival of myotubes, structural development of the excitation–contraction coupling apparatus and neonatal myosin heavy chain expression, Biomaterials 30 (29) (2009) 5392–5402.
[111] M. Das, J.W. Rumsey, N. Bhargava, M. Stancescu, J.J. Hickman, A defined long-term in vitro tissue engineered model of neuromuscular junctions, Biomaterials 31 (18) (2010) 4880–4888. http://dx.doi.org/10.1016/j.biomaterials.2010.02.055.
[112] X. Guo, S. Spradling, M. Stancescu, S. Lambert, J.J. Hickman, Derivation of sensory neurons and neural crest stem cells from human neural progenitor hNP1, Biomaterials 34 (18) (2013) 4418–4427. http://dx.doi.org/10.1016/j.biomaterials.2013.02.061.
[113] B. Gerin, S. Dell'Aiera, L. Richert, S. Smith, H. Chanteux, Assessment of cytochrome P450 (1A2, 2B6, 2C9 and 3A4) induction in cryopreserved human hepatocytes cultured in 48-well plates using the cocktail strategy, Xenobiotica 43 (4) (2013) 320–335. http://dx.doi.org/10.3109/00498254.2012.719088.
[114] I.K. Zervantonakis, S.K. Hughes-Alford, J.L. Charest, J.S. Condeelis, F.B. Gertler, R.D. Kamm, Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function, Proc. Natl. Acad. Sci. 109 (34) (2012) 13515–13520.