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Organs-on-a-chip for drug discovery

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The current drug discovery process is arduous and costly, and a majority of the drug candidates entering clinical trials fail to make it to the marketplace. The standard static well culture approaches, although useful, do not fully capture the intricate *in vivo* environment. By merging the advances in microfluidics with microfabrication technologies, novel platforms are being introduced that lead to the creation of organ functions on a single chip. Within these platforms, microengineering enables precise control over the cellular microenvironment, whereas microfluidics provides an ability to perfuse the constructs on a chip and to connect individual sections with each other. This approach results in microsystems that may better represent the *in vivo* environment. These organ-on-a-chip platforms can be utilized for developing disease models as well as for conducting drug testing studies. In this article, we highlight several key developments in these microscale platforms for drug discovery applications.

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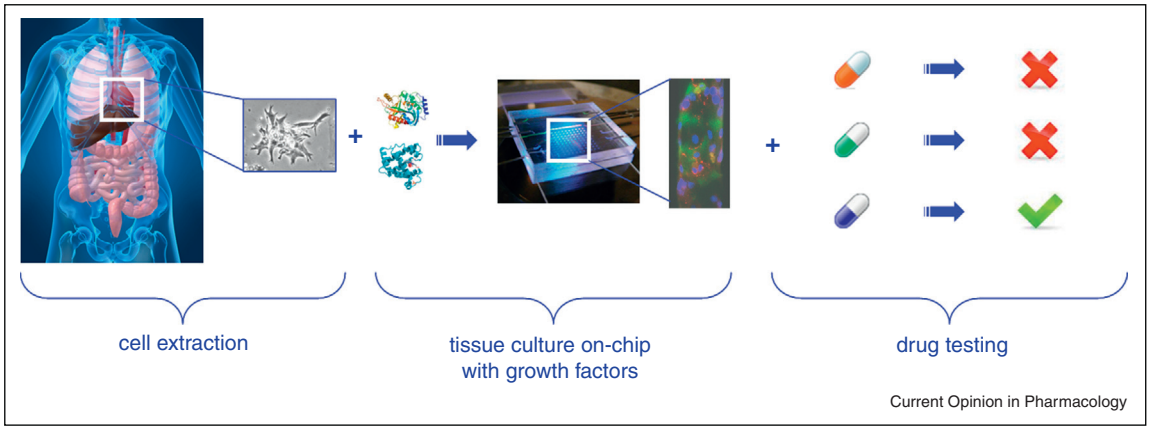
Introduction

The conventional drug discovery process is long and costly, as the majority of the drug candidates tend to fail during the clinical trials. A major reason for such a low success rate is our inability to predict the toxicity and efficacy of drugs before expensive human clinical trials. For example, *in vivo* the tissues reside in a dynamic environment, which is continuously perfused with blood involving interactions between cells and organs. In addition, the cells or the tissues within the body are constantly being stimulated by chemical, mechanical and electrical cues. Finally, human cells are known to respond differently to chemicals compared to animal cells and therefore new, efficient systems must utilize human tissues to be able to predict complex responses. Novel technologies at the interface of tissue engineering and microfluidics are emerging as candidates that may be able to aid this field and accelerate the drug discovery process.

Adopted from the traditional semiconductor industry, microfabrication and microfluidic technologies are powerful approaches to create small structures for a variety of applications in biotechnology. In drug discovery, microscale platforms allow precise delivery of fluids with reduced reagent volumes and can be utilized for high-throughput screening [1–4]. Microengineering technologies can also be used for fabricating tissue-like structures that mimic the natural complexity of tissues [5]. Such microscale tissue platforms may be useful in recreating the intricacies of the *in vivo* environment with microscale precision and can also provide chemical, electrical, or mechanical cues representative of the living environment. For example, microscale perfusion bioreactors (devices enabling manipulation of biological materials, such as proteins, cells, or tissues) can be generated through the use of already available microfluidic systems for loading, manipulation, and analysis of a sample [6]. These systems present a dynamic environment for cells, and can be used for drug toxicity studies. Furthermore, the fabrication of metal electrodes on ultra thin stretchable substrates can be used to record or stimulate signals from brain slices [7]. Additionally, on-chip sensors are being developed to monitor tissue viability and functionality in real-time [8]. The technology to create constructs with microscale resolution also provides an opportunity to precisely manipulate a certain number of cells within these platforms [9].

The creation of functional tissue constructs on-chip with an ability to control the cellular microenvironment presents numerous opportunities in basic biology, tissue engineering and drug screening studies. Moving beyond static cultures, this emerging and exciting field termed 'organs-on-a-chip' provides opportunities to probe the cellular behavior against a plethora of stimuli [10–12]. These systems can further be utilized to create disease models, they can be perfused to create dynamic culture environments (Figure 1), and can be exposed to gradients of drugs — all on the same platform [13] (Figure 2). In this review, we highlight several key papers in the organ-on-a-chip field, namely the heart, the lung, and the intestine. Although studies are being conducted to generate tissues representing most, if not all organs in the human body — from the eye and the skin, to the blood–brain barrier and neuronal tissue, to cartilage and bone tissues — we have

Fig. 1



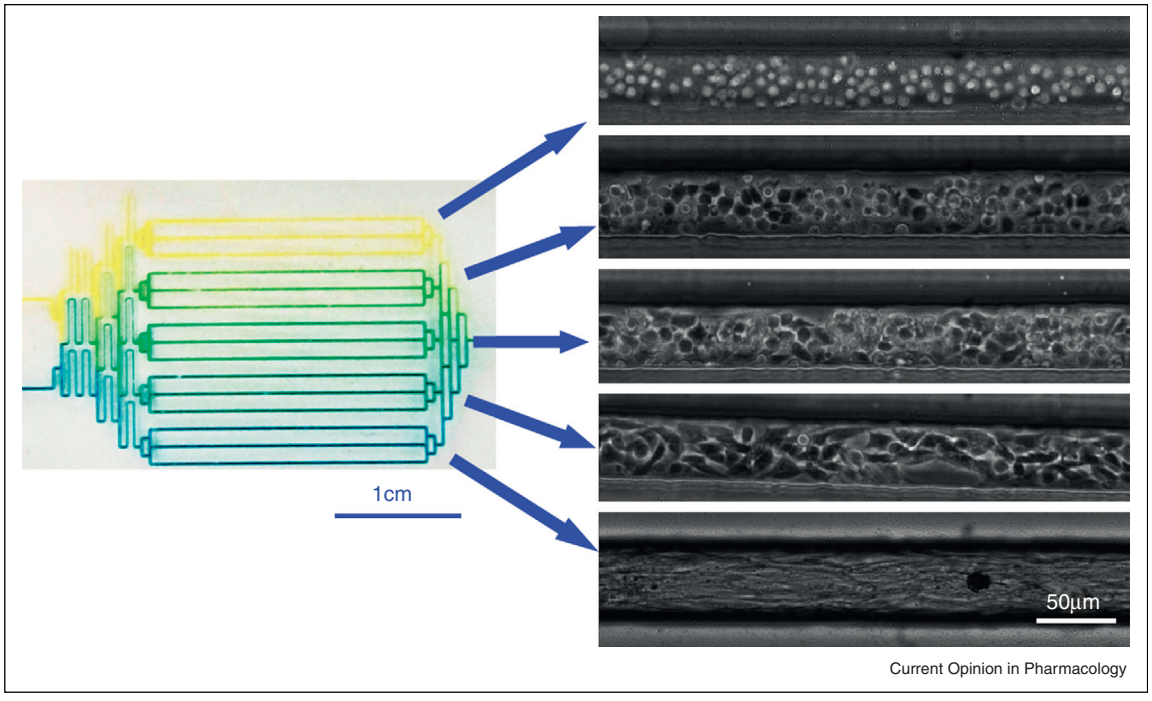
Concept behind organ-on-a-chip modules for drug metabolism and toxicology studies. The tissue model is engineered using microscale technologies and begins with the extraction of cells from a living organism or with cells from a cell line (left). The cells are introduced into a microfluidic chip together with growth factors and other biocompatible molecules for tissue culture, which can be visualized using immunostaining (center). The functional tissue can then be exposed to one or more drugs of interest, and is further analyzed with respect to expressed proteins and overall health, indicating viability and functionality of the tissue (right).

chosen to focus on recent organs-on-a-chip that have utilized efficient microfluidic solutions.

Heart

Heart disease is responsible for 1 out of 4 of all deaths in the United States, roughly 600 000 cases every year [14]. Hence, research on cardiac drug development, side effects of drugs and the interactions between multiple drugs has been a strong focus in the medical community [15–17]. It is also important to detect any cardiotoxic effects of medications early in the drug development process, as their withdrawal from the market (e.g., Terfenadine,

Fig. 2



Example of a microfluidic organ-on-a-chip for tissue culture. The top view of the device (left) shows an array of parallel microfluidic channels, which can be loaded with different cells, growth factors, or drugs (here visualized with different food dyes). The phase contrast images on the right exemplify the results of different cell culture conditions (rat cardiofibroblasts) on the same chip.

Astemizole, Grepafloxacin, Cisapride, Thioridazine) can cost billions of dollars. Microscale on-chip solutions representative of the *in vivo* conditions therefore offer an attractive platform to conduct cost-efficient and time-efficient drug assays before the clinical trials [18,19].

A recent example of such a microscale solution has been offered by Parker and colleagues [20•], who succeeded in engineering functional cardiac tissue on a flexible substrate using microfabrication technology. Heart muscle cells were cultured onto small tissue strips, where they began to align with each other and beat in unison under certain conditions. Then they were exposed to drugs and electrically stimulated. The strips were seeded with a small number of cells, which enabled excellent control over the tissue development. Observing the resulting contractile response of the tissue enabled the researchers to quantify the cardiotoxic effects of the drugs. For example, the addition of epinephrine (adrenaline) led to a fourfold increase in the beating rate of the heart tissue, indicating that this setup could be used to determine the appropriate dose of a medication for treatment. Although this platform is used for static culture, it would be straightforward to merge it with a simple perfusion system and enable dynamic conditions. This will also permit time-dependent and concentration-dependent drug tests. In addition, on-chip measurements of the contractile stresses and the electrical activity of the tissue serve to generate a more complete picture of the tissue response. Effectively, this heart-on-a-chip device offers researchers a view of a healthy or damaged cardiac tissue.

Aubin *et al.* [21] have studied the three-dimensional (3D) functionality of cardiac tissues using microscale hydrogels. Cardiomyocytes were encapsulated inside hydrogels with different micropatterns and shown to align and form fiber-like structures most efficiently when constrained inside long, narrow hydrogel units, an outcome that was made possible with the application of microfabrication technologies. These results indicated that microscale scaffolds could be engineered to mimic the size and shape of individual cardiac fibers *in vivo*. Once cardiac tissue can be constructed in this fashion in the laboratory, it can be applied to conduct drug tests, as done by Grosberg *et al.* [20•]. In another approach, Giridharan *et al.* [18] cultured embryonic cardiac cells inside a microscale cylindrical bioreactor and exposed them to pulsatile flow and varying strains to mimic the cardiac cycle in the left heart ventricle. Video recordings and a pressure sensor enabled the researchers to measure the heart rate, systolic and diastolic pressures, and to model common ailments like hypotension and hypertension as well as tachycardia and bradycardia. It would be straightforward to spike the cardiac medium with a drug of interest and observe its effect on tissue function.

In the future, devices like these — merging electrical, chemical, and mechanical impulses with a tissue culture reactor — could be utilized for studies of human stem-cell derived cardiac cells, specifically to predict the toxicity of drugs and environmental agents on the human heart. Equally important, they may enable the development of more efficient treatments for common diseases such as arrhythmia. This line of research might potentially lead to personalized medicine, which would allow for drug testing on patients' own cells to maximize the success of treatment.

Lung

The lung is also subject to a range of diseases, such as asthma, chronic bronchitis, emphysema, and cancer. According to the American Lung Association, close to 9% of all adults in the US suffer from asthma or another condition affecting the lungs [22]. To better understand the changes in lungs due to injuries or cellular decay, or even just the mechanics of breathing, it is vital to study the tissue *ex vivo*. On-chip solutions utilizing microfluidics have enabled the generation of functional lung tissues mimicking certain aspects of the *in vivo* structural complexity.

Recently, physiologically functional lung-on-a-chip devices are being developed for studying diseases such as pulmonary edema. In this disease, intravascular fluid passes to and accumulates in the lung alveoli (hollow air sacs), leading to shortness of breath. Huh *et al.* [23] used a microfluidic setup to first generate the alveolar–capillary interface of the living human lung-on-chip and then impose breathing movements on the tissue, by repeatedly stretching the cultured cells. The application of microfluidics enables these well-controlled mechanical cues, as well as a dynamic perfusion culture system for long-term studies. This device was used to reproduce the pulmonary edema observed in human cancer patients treated with interleukin-2 (IL-2). On-chip studies revealed that a crucial role was played by the mechanical forces associated with physiological breathing motions. Using this model, Huh *et al.* [24••] were also able to identify an inhibitor of the IL-2 induced pulmonary edema, acting on a particular ion channel expressed by the human lung which was activated by mechanical strain. These data suggest the relevance of the lung-on-a-chip model [24••]. Extending this work, the same or a similar setup could be used to develop other human disease models, identify new medications and determine the toxicity of existing ones. Interestingly, the emergence of alveoli was also observed by Douville *et al.* [25], who investigated the effects of solid and fluid mechanical stresses on cell behavior in their microfluidic lung model.

The proposed lung-on-a-chip model focuses on the capillary-alveolar interface, yet some lung diseases may require a much more complex, 3D model of the organ

that involves a vascular system for adequate supply of nutrients and oxygen to the tissue. Kniazeva *et al.* [26] have recently applied microfluidics to combine capillary channels for blood delivery with a large membrane for the exchange of oxygen. The resulting device contained interdigitated layers of blood and oxygen filled channels that can be stacked to produce a 3D lung architecture, which could not be generated using standard macroscale approaches. Merging this vascular network with the stretchable platform of the lung-on-a-chip could potentially lead to the first 3D engineered model of human lung tissue, and as a result to an *in vitro* model of the whole organ.

Intestine

The gastrointestinal (GI) tract is also susceptible to a range of chronic conditions such gastroenteritis, Crohn's and Celiac diseases. Despite its importance, microfabricated devices mimicking the GI tract have only recently become a source of interest. This is partially due to the highly complex nature of the GI tract, including its intricate topography, the contractile motion of the intestines, and the myriads of naturally present bacteria [27–29]. However, microfluidics offers yet again an efficient, functional model of the relevant tissue. Kim *et al.* [30••] utilized a device structure similar to the previously described lung-on-a-chip device, in order to mimic the flow inside and peristaltic motion of the intestines while culturing the cells. This cyclic stretch of the cell layer — an important mechanical cue to the cells — was found to greatly accelerate the cell alignment and proliferation and to enable the formation of villi, the columnar topography specific to the intestinal walls. Alternately, the villi could be incorporated into the GI scaffold topography *a priori*, by various molding procedures, as shown by Sung *et al.* [31]. Importantly, a perforated membrane was incorporated between the cell layer and a second microfluidic channel mimicking the tissue behind the endothelial walls. Kim *et al.* studied the relevance of microbes to the structural integrity and health of the intestinal tissue by quantifying the permeability of the membrane layer. It was shown that a bacillus common to the human GI flora helped strengthen the intestinal epithelial barrier. An overgrowth of the bacillus could damage the tissue, but on this chip any excess bacteria were removed by the flow of liquids, similar to the *in vivo* case. This type of data could be helpful in developing new treatments for conditions such as appendicitis, which often require surgical intervention. Furthermore, introducing different types of bacillae into the gut-on-a-chip could shed light on absorption anomalies like lactose or gluten intolerance.

In this context, Ramadan *et al.* [32] have recently studied the effects of dairy products on the immune functions inside the GI tract using their microfluidic intestine-on-a-chip titled NutriChip. Here, epithelial cells were co-cultured with immune cells, separated by a permeable membrane. This enabled the researchers to study the response of the immune cells to any materials (e.g., dietary proteins) that passed through the epithelial layer. Specifically, the inflammatory effects of lipid polysaccharides were investigated, as well as the consequences of adding dairy nutrients to this microfluidic GI system.

Aside from nutrikinetics, the absorption mechanisms in the intestines are crucial in pharmacokinetics and, ultimately, in drug development. Namely, orally administered drugs are absorbed in the small intestine, through a mucous layer and a layer of epithelial cells. Hence, a controlled *in vitro* environment, such as the gut-on-a-chip, could serve to screen drugs for their absorption and potential toxicity at the intestinal level.

Conclusions and outlook

In this review, we have provided a glimpse into some of the latest developments in the organ-on-a-chip field with the intent to create biological functions on-chip for disease modeling and drug discovery applications. Among the approaches discussed, the heart-on-a-chip project is enabled by cultivating cardiomyocytes on-chip, where researchers observed increases in beating rates in response to drugs. In another application, using a stretchable chip, the breathing movements of the lung were mimicked, which was used to develop an *in vitro* model of the pulmonary edema. Furthermore, by applying mechanical forces to a type of GI cells during culture, the formation of columnar epithelium recapitulating the structure of intestinal walls was observed. The addition of microfluidic channels to these platforms not only provides a perfusable dynamic environment, but also mediates the delivery of drugs to these cell constructs in a controllable manner. In addition, one can also couple on-chip sensors to these systems to enable real-time monitoring of cellular viability and function.

Early work in the organ-on-a-chip area looks promising. We believe that we are seeing unprecedented growth in this area with the introduction of novel platforms that model selected biological functions or diseases on-chip. In time, the proliferation and standardization of these approaches will provide a new set of tools for users not only to create and study disease models, but also to conduct drug screening in a rapid and efficient manner.

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