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Microfluidics-Based in Vivo Mimetic Systems for the Study of Cellular Biology

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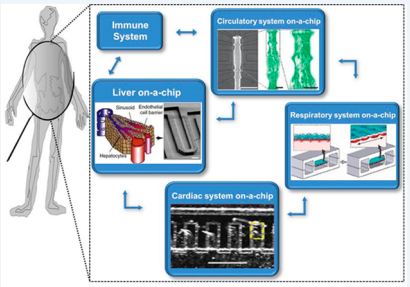
CONSPECTUS: The human body is a complex network of molecules, organelles, cells, tissues, and organs: an uncountable number of interactions and transformations interconnect all the system's components. In addition to these biochemical components, biophysical components, such as pressure, flow, and morphology, and the location of all of these interactions play an important role in the human body. Technical difficulties have frequently limited researchers from observing cellular biology as it occurs within the human body, but some state-of-the-art analytical techniques have revealed distinct cellular behaviors that occur only in the context of the interactions. These types of findings have inspired bioanalytical chemists to provide new tools to better understand these cellular behaviors and interactions.



Inset images reproduced from Huh et al. *Science* **2010**, *328*, 1662–1668; Agarwal et al. *Lab Chip* **2013**, *13*, 3599–3608; Lee et al. *Biotechnol. Bioeng.* **2007**, *97*, 1340–1346; and Gunther et al. *Lab Chip* **2010**, *10*, 2341–2349.

What blocks us from understanding critical biological interactions in the human body? Conventional approaches are often too naïve to provide realistic data and in vivo whole animal studies give complex results that may or may not be relevant for humans. Microfluidics offers an opportunity to bridge these two extremes: while these studies will not model the complexity of the in vivo human system, they can control the complexity so researchers can examine critical factors of interest carefully and quantitatively. In addition, the use of human cells, such as cells isolated from donated blood, captures human-relevant data and limits the use of animals in research. In addition, researchers can adapt these systems easily and cost-effectively to a variety of high-end signal transduction mechanisms, facilitating high-throughput studies that are also spatially, temporally, or chemically resolved. These strengths should allow microfluidic platforms to reveal critical parameters in the human body and provide insights that will help with the translation of pharmacological advances to clinical trials.

In this Account, we describe selected microfluidic innovations within the last 5 years that focus on modeling both biophysical and biochemical interactions in cellular communication, such as flow and cell−cell networks. We also describe more advanced systems that mimic higher level biological networks, such as organ on-a-chip and animal on-a-chip models. Since the first papers in the early 1990s, interest in the bioanalytical use of microfluidics has grown significantly. Advances in micro-/nanofabrication technology have allowed researchers to produce miniaturized, biocompatible assay platforms suitable for microfluidic studies in biochemistry and chemical biology. Well-designed microfluidic platforms can achieve quick, in vitro analyses on pico- and femtoliter volume samples that are temporally, spatially, and chemically resolved. In addition, controlled cell culture techniques using a microfluidic platform have produced biomimetic systems that allow researchers to replicate and monitor physiological interactions. Pioneering work has successfully created cell−fluid, cell−cell, cell−tissue, tissue−tissue, even organ-like level interfaces. Researchers have monitored cellular behaviors in these biomimetic microfluidic environments, producing validated model systems to understand human pathophysiology and to support the development of new therapeutics.

■ INTRODUCTION

While understanding human biology is essential for medical advances, measurement limitations obscure critical details. The human body is a complex network of biophysical (flow, morphology, and gradients) and biochemical (molecules, matrixes, and cells) components, and human pathophysiology is a result of those components acting both individually and synergistically. While significant research has been performed on the individual components of the system, understanding the interactions occurring in the human body has been largely limited because of technical barriers. Conventional approaches are typically either quick in vitro assays on isolated cells in a simplified environment or slow in vivo assays using animal models to account for the complex nature of the immune system; unfortunately, neither of these approaches provide clear insights into the cell−cell interactions occurring inside the human body. As such, there is a clear demand for bioanalytical chemists to provide new principles and tools to bridge the gaps in our understanding.

Recent advances in technology provide opportunities to overcome the limitations of traditional methodologies: any new analytical platform for this purpose must (1) facilitate incorporation of human cellular components, while (2) resolving the behavior of individual components with (3) quick, practical, affordable, and reliable assays. Microfluidic platforms, miniaturized devices with micrometer-sized channels and chambers, are good candidates for all of these requirements.[1−6] This Account presents recent innovations in

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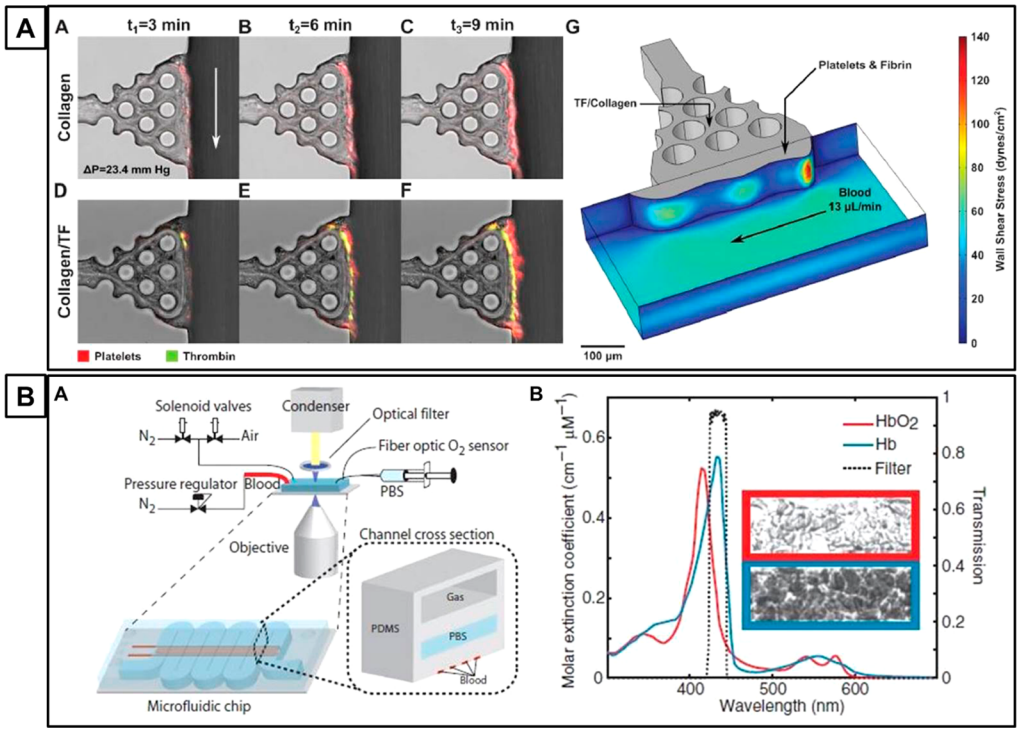

Figure 1. (A) Whole blood perfusion enables reproducible development of thrombi at the interface between collagen and blood.[15] Platelets and thrombin are shown in red and green, respectively, on either collagen (a–c) or collagen/tissue factor scaffolds (d–f). Time-dependent resulting structures at the clot boundary were simulated in COMSOL (g). Reproduced from ref 15 with permission. (B) Three layer microfluidic platform for the study of sickle cell blood flow conductance: artificial capillary layer for blood flow, a hydration layer, and a gas reservoir.[16] A digital pressure regulator and two solenoid valves were utilized for device operation, and oxygen concentration in the gas reservoir was measured using a fiber optic probe (a). Deoxygenated red blood cells show a shift in the hemoglobin absorption peak, and in the inset, representative images of oxygenated (transparent) red blood cells and deoxygenated (dark) red blood cells are presented (b). Reproduced from ref 16 with permission.

microfluidics for fast in vitro assay solutions that mimic in vivo environments.

MICROFLUIDICS IN THE STUDY OF CELLULAR BIOLOGY

Because of significant advances in microfabrication technologies from the semiconductor industry, microfluidic channels and chambers can be generated in nearly any geometry in a variety of materials with opportunities for high-throughput and multiplex analyses. Even within the limited context of biological applications, microfluidic channel geometries vary from simple straight channels to complex 3-dimensional (3D) structures,[1,5] and the device material varies from glass and silicon, to polymers, such as polydimethylsiloxane (PDMS) and hydrogels.[5,7] Hybrid devices have been created that incorporate nanoscale metal structures (mostly gold and silver in biological applications) into the microfluidic device to facilitate molecular detection.[6−8] Also, various surface chemistries have been adapted to create cell-friendly surfaces, enabling the study of either short-term (seconds/minutes) or long-term (hours/days) cellular behaviors.[1,5,7,9] As such, microfluidics has been intensively employed in various fields of chemical, biological, pharmaceutical, and medical studies in recent years.[1−9] The two main focus areas of microfluidics-based approaches to better understand human biology are (1) providing high-throughput single cell analysis to measure heterogeneity of cellular responses[3,10] and (2) providing an in vivo mimic to account for the dynamic and complex nature of the human body.[1,5,9,11] This Account focuses on the latter where significant breakthroughs in the field have been made by exploiting in-vivo-mimicking microfluidic assays and have revealed distinct cellular behaviors that are obvious only in environments that model the true in vivo environment.[1,5,9,11] For example, it has been reported that neutrophils on a fibronectin-coated surface behave differently than neutrophils cultured on endothelial cells.[12] Also, cultured epithelial cells show more in-vivo-like molecular transport behaviors when they are exposed to flow than when they are kept in a static environment.[13] The focus areas in this Account will describe microfluidic applications developed within the last 5 years that (1) model biophysical aspects of the immune system (e.g., flow), (2) model biochemical aspects of the immune system (e.g., cell–cell networks), or (3) combine higher level biological networks (e.g., organ on-a-chip and animal on-a-chip). These microfluidic approaches facilitate both fundamental understanding of human pathophysiology and therapeutic development.

RECONSTITUTION OF DYNAMIC ASPECTS OF THE IN VIVO

Not surprisingly, the biochemical and biophysical microenvironment influence cellular adhesion, morphology, and differentiation, and microfluidic methods make it possible to control such factors. For example, Nakao et al. have recently shown achievement of a hepatic-cord-like structure of hepatocytes simply by reconstituting the tissue–tissue interface.[14] Also, simple exposure of epithelial cells to physiological shear stress in the presence of drugs like vasopresine and aldosterone induced in vivo levels of water/salt transport by the epithelial cells.[9,13] As such, it is important to consider the influence of the microenvironment on cellular behaviors.

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Among the various possible microenvironmental aspects, this section focuses on replicating in vivo flow characteristics within an in vitro experimental system. Fluid flow is ubiquitous in the human body and facilitates connections among all the components of the human body; for example, flow-induced mechanical forces are known to impact the cellular cytoskeleton, morphology, and adhesion.[15−18] One of the natural strengths of microfluidics is accurate control over fluid behaviors and easy adjustment of flow-induced forces across a range of physiologically relevant levels, all with high throughput and small sample volumes.[3,7,10] Conventional platforms, such as flow chambers, can explore flow-induced characteristics, but the flow control is spatially and temporally limited. In the following section, in vitro microfluidic studies that focus on flow-induced aspects of the human body will be concisely described to demonstrate the utility of microfluidics. In the human body, flow forces can either induce a biological response or can be the actual biological response to a stimulus; as will become clear below, microfluidics provides simple but powerful ways to consider both aspects of flow and broaden our understanding of critical cellular processes occurring inside the human body.

Replication of Flow-Induced Cellular Responses

Let us begin with a simple example of blood clotting, an important process to prevent fatal damage to blood vessels. What causes blood clotting? Clinical and macroscopic answers for this question can vary, but microscopically, it is a result of biochemical/biophysical interactions between the vessel wall and circulating cells (mostly platelets and red blood cells). This process starts with platelets adhering to and aggregating at the site of injury on the blood vessel wall. While this process remains insufficiently characterized, it has been proposed that flow plays a critical role in platelet adhesion/aggregation.[4,17,18] As such, one obvious approach to understand blood clotting is to study the impact of flow on the platelet adhesion/aggregation process. Gutierrez et al. developed a microfluidic platform to test the hypothesis that flow-induced shear stress is the cause of different platelet adhesion levels to the extracellular matrix.[17] This work investigated platelet adhesion to several physiologically relevant extracellular matrixes: von Willebrand factor, fibrinogen, and collagen, under a wide range of shear stress levels. Their on-chip investigation revealed that intra- and extracellular platelet adhesion receptors had different impacts on platelet adhesion under different shear stress levels. Specifically, this study revealed that the inside-out signaling of integrin αIIbβ3 varied based on extracellular shear stress levels, greatly influencing platelet adhesion to the aforementioned extracellular matrices. The high-throughput nature of this study allowed study of platelet interaction with various extracellular matrices under several physiologically relevant shear stress conditions, and enabled easy monitoring of intracellular signaling on the same device. Similar microfluidic approaches have been used for various related studies.[16−20] In fact, our lab recently published work where cellular biology relevant to blood clotting events are systematically studied using simple microfluidic platforms with controlled flow.[19] Our studies investigated the impact of nanoparticles on platelet adhesion under varying flow conditions, and revealed flow-dependent platelet adhesion in both cases.[19] From these examples, it is clear that microfluidic in vivo mimetic systems enable evaluation of cellular processes in physiologically relevant environments, and the findings from such studies give critical insight into fundamental biological processes. Similar devices and studies could be easily adapted to other biological models, such as leukocyte rolling, and could also contribute to ex vivo studies by connecting the device to, for example, the artery of an animal.

Evaluation of Cellular Response-Caused Changes in Flow

The previous examples clearly show how microfluidics can be used to evaluate flow-induced causes of cellular processes within human body. Similar devices can be used to measure flow changes as an outcome of biological events. For example, when blood clotting becomes abnormal, thickened blood vessel walls increase the blood pressure which leads to further blood clotting; there is a positive feedback loop between flow-induced pressure and blood clotting. Muthard et al. have recently reported a microfluidic platform where they monitored flow-generated shear stress and correlated it to clot size/composition.[15] On-chip visualization of thrombosis facilitated assessment of clot formation, structure, size, composition, and the relationship between platelet adhesion and thrombin generation at various shear stress conditions. Their approach clearly provides mechanistic understanding of the positive feedback loop between flow-induced shear stress and blood clotting. In another example, Wood et al. have exploited microfluidics to study a sickle cell disease-relevant hypothesis that deoxygenated red blood cells ("sickled" cells) flow slowly in the bloodstream, enhancing probability of cell−cell contact, and thus, creating a positive feedback loop for further blood clotting.[16] To test their hypothesis, they developed a comprehensive microfluidic platform that facilitated control of red blood cell deoxygenation in blood and monitored the velocity of the blood stream flow. Their methodological innovation was the incorporation of simultaneous manipulation of oxygen gas and blood fluid within the device and realization of simultaneous measurement of both cellular morphology and flow velocity. With this innovative microfluidic approach, they could identify individuals with mild or severe sickle cell disease, and even further, could predict patients' response to therapies. In these two examples, the former approach exemplifies how microfluidics can broaden our understanding of critical biophysical characteristics while the latter exemplifies the potential of microfluidic diagnostics. Such approaches benefit early detection of disease, monitoring the progression, and even the prediction of patients' responses to treatments and will broaden our understanding of fundamental biochemistry occurring in the human body.

There are many examples of microfluidic studies of flow effects in biology beyond those presented above. Recently, Korin et al. developed a microfluidic approach mimicking in vivo flow to assess drug delivery from therapeutic nanoparticles to a designated site.[20] They developed nanoparticle aggregates that disassemble at a certain shear stress level and tested delivery of antithrombotic drugs, validating a novel therapeutic to justify future preclinical studies. As in the work exemplified above, microfluidics provides a simple but powerful approach for cellular biology studies to account for the flow-induced biophysical characteristics of the human body. In addition to considerations of flow, microfluidics allows experimentalists to better simulate the in vivo biochemical microenvironment by incorporating various biomolecules or cell populations.[12,21] The complexity of such biochemical features of the in vivo milieu have been hindering our understanding of human biology gleaned from in vitro studies; as such, microfluidic in vivo

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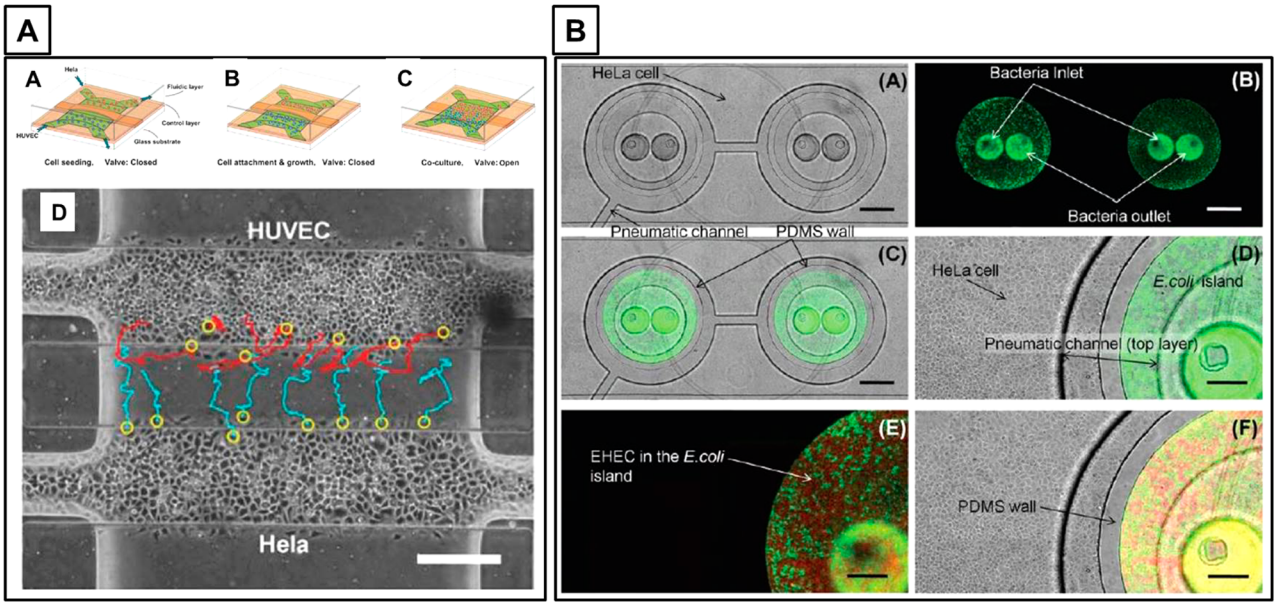

Figure 2. (A) Schematic of the microfluidic coculture device (a–c) with a representative image of HeLa and HUVEC cells cultured in the microfluidic device (d, scale bar: 300 μm). Reproduced from ref 2 with permission. (B) HeLa cell–bacteria coculture system in a microfluidic device. HeLa cell monolayer (a), GFP-expressing *Escherichia coli* BW25113 localized in the bacterial islands (b), coculture of HeLa cells and *E. coli* BW25113 (c), close-up view of the coculture (d), RFP-expressing EHEC and GFP-expressing *E. coli* BW25113 (e), and transmitted, green, and red fluorescence images in the device (f). Scale bar in panels a–c represents 500 μm, and scale bar in panels d–f represents 200 μm. Reproduced from ref 25 with permission.

mimetic platforms possess great potential for facilitating better understanding of human biology.

RECONSTITUTION OF IN VIVO MOLECULAR AND CELLULAR COMPLEXITY

While flow is a critical biophysical parameter that influences cells, biochemical signals, including neurotransmitters, hormones, and chemokines among others, are presented in dynamic, complex mixtures to influence cellular behavior. Conventional methods to experimentally address such complexities has been largely limited to the use of expensive, slow in vivo assays that are not necessarily relevant to human physiology; however, microfluidics provides a unique gateway where such molecular and cellular interactions can be observed in a temporally, spatially, and chemically resolved manner. During in vivo cell–cell interactions, delivery of chemical mediators can be achieved by convective motion of the chemical mediator-containing fluid, diffusion, or direct delivery upon physical contact, all of which can be simulated in a microfluidic device. For example, Skelley et al. utilized a novel microfluidic device to observe fusion efficiency in a coculture of fibroblasts, myeloma cells, and embryonic stem cells.[22] They created arrays of individual cell traps that allowed controlled cell–cell contacts, that is, controlled chemical mediator delivery by physical contact. Their microfluidic approach significantly improved cell–cell pairing efficacy when compared to a conventional meticulous cell pairing, and the critical factors in the fusion event between targeted cell types, such as size matching or flow-induced forces, were closely analyzed in a high-throughput manner. Like this work by Skelley et al., there have been significant research efforts to realize microfluidic mimetics of in vivo molecular and cellular interactions, and these efforts have identified several critical aspects of human biology that were not easily obtainable by conventional methods. As such, this section provides studies where such "interactions" have been considered; to broaden readers perspectives on the potential of this field, studies were categorized into two critical biological models.

Cell–Cell Interactions in Cancers

In cancer, studies of circulating tumor cells (CTCs) are popular because many believe CTCs are the first sign of impending tumor metastasis.[2,23,24] There are many open questions about how the CTCs become activated, find a susceptible opening in the endothelium, and traverse the endothelium to induce a subsequent tumor. Accordingly, tumor cell–endothelial cell interactions and chemical mediator exchange between those cells are of significant interest. Microfluidic platforms provide a simple but powerful tool to study these interactions. For example, several microfluidic approaches that incorporated semipermeable membranes or pressure-driven microfluidic valves have realized a coculture system of tumor cells and endothelial cells.[2,23,24] In the microfluidic system by Zheng et al., chemical mediator delivery between tumor cells and endothelial cells is closely monitored without interference from actual physical contact.[2] The microfluidic platform in this study is equipped with pressure-driven microfluidic valves as a physical barrier, and upon valve opening, exchange of chemical mediators between tumor cells and endothelial cells begins, and the device allows real time investigation of cellular migration according to the chemical interactions. In another example, Hsu et al have recently investigated the paracrine loop between cancer cells and fibroblasts, another connective tissue cell that cancer cells interact with, using a microfluidic device.[24] They tested and affirmed the hypothesis that cancer cell-fibroblast interactions induce fibroblast differentiation into another subphenotype known as myofibroblasts; in fact, the device allowed them to identify growth factor TGF-ß1, a chemical mediator, as a critical component of this transformation. A similar microfluidic approach was reported by Ma et al. to model the complex microenvironments surrounding fibroblast-

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cancer cell interactions.[23] By enabling chemical communication, without physical contact, between tumor cells and embryonic lung fibroblast cells or mature epithelial cells, this study revealed differences between epithelial cells and embryonic fibroblasts in responding to tumor cells. All the above microfluidic platforms enable (1) manipulation of precise chemical gradients within the device, (2) controlled coculture of cancer cells and stromal or connective tissue cells, and (3) most importantly, temporally, spatially, and chemically resolved analysis on individual components of the experimental system. In addition, the device used by Zheng et al. exemplifies the versatility of microfluidics, numerous experiments were performed using the same device design, maintaining uniformity for result comparison and minimizing the time required for device engineering.[2] In the device employed by Hsu et al., the valuable multiplexing capability of microfluidics is demonstrated based on their ability to incorporate multiple cell types into a single device.[24]

Cell–Cell Interactions in Bacterial Infection

As in cancer, cell–cell interaction in bacterial infection is critical but not well characterized. Microfluidic coculture of multiple cell types provides opportunities to bridge this gap. Epithelial cells line the major cavities in the body and make up some of the most vital and resilient organ systems. They come in contact with the outside world much more frequently than endothelial cells and therefore, are one of the most relevant model cells to coculture with bacterial pathogens. In this spirit, Kim et al. studied the interaction between *Escherichia coli* and HeLa cells in a microfluidic device controlled by pressure-driven microfluidic valves.[25] As above, the microfluidic device maintained physical separation between the bacterial and epithelial cells using pneumatic control so that a definite "time zero" could be established and time-resolved infiltration of bacteria into the HeLa cells could be measured while also preventing premature epithelial cell death that otherwise occurs after an exposure time of only six hours. Through the use of biofilm islands, this group demonstrated that bacteria display a spatial bias to colonization, gathering insight on how *E. coli* outcompetes commensal bacteria in the gastrointestinal tract. Another example of a microfluidic device utilized in the study of epithelial coculture with bacteria by Hong et al.[26] investigated the use of bacteria in targeting cancer. A device utilizing a large middle chamber separated from separate side channels by a collagen layer allowed for the coculture of both cancer cells and normal cells. Bacteria were introduced through the middle chamber and the chemotactic behavior was observed. The primary goal was to observe the activity of *Salmonella typhimurium* when exposed to the competing chemokine gradients secreted by normal hepatocytes and hepatic carcinoma. Hong et al. demonstrated that *S. typhimurium* shows a clear preference for cancer hepatocytes over normal hepatocytes, introducing them as a possible candidate in bacteria-driven cancer targeting.[26]

Microfluidics is clearly a simple and powerful way to consider molecular and cellular complexity in vivo. For coculture studies in particular, microfluidics use pressure-driven valves or semipermeable membranes to enable the critical separation among cultured cell types to prevent interferences and evolution of subphenotypes in the cultured cells. This capability provides opportunities to examine cellular function at a microenvironmental level, even in the context of cell–cell interactions, that is otherwise unachievable. Another way microfluidics can facilitate microenvironmental control is in the creation of tissue spheroids, sphere-shaped, self-assembled tumor microenvironments closely mimicking avascular tumors and micrometastases. Conventional methodologies are unable to form uniform and stable spheroids in high yield. The innate microfluidic control over fluid flow and placement of cells provides opportunities to address such issues.[27] In addition, the accessibility of various on-chip detection schemes facilitates in-depth characterization of biochemical events occurring in spheroids.[26] In fact, one can go beyond coculture or spheroid creation to more complex organ mimetics to provide methods for drug screening or organ transplant assessment. Monitoring the potential impact of drugs or the efficacy of transplantation adds several additional demands on microfluidic device design, including consideration of 3-D structure, geometries, stiffness, permeability, and density of the included cell aggregates. Microfluidics opens opportunities to consider nearly any aspect of the in vivo environment and achieve new understanding of human biology that cannot be achieved with traditional methods.

■ ORGANS-ON-A-CHIP

So far, this Account has described achievements in the field where either biophysical or biochemical aspect of in vivo has been considered; however, despite significance of those individual aspects, accurate prediction of biological events in human body requires insight beyond simple cell-flow and cell–cell interactions probed in the aforementioned devices; thus, a new segment of microfluidic research has emerged to pursue more complex systems known as organs-on-a-chip. The goal of an organ-on-a-chip is reconstitution of 3D human organs on a microfluidic platform with precise control over chemical, physical, and biological characteristics of the target system.[34−39]

For the purpose of recapitulating physical environments, perfusion culture conditions are applied to generate the mechanical forces that cells experience in living organisms. Microfluidics provides precise control over various extracellular matrices to better mimic cell-matrix interactions and support formation of 3D cell layers in in-vivo-relevant structures. The integration of microelectrodes into microfluidic devices, the employment of technologies called microelectro-mechanical systems, is critical for several organ-on-a-chip examples, such as heart tissues and neuronal systems, as these organ systems frequently require electrochemical stimulation. Computational simulation is also critical in realizing organ-on-a-chip systems to prepare appropriate flow- and structure-induced mechanical characteristics. Incorporation of organ functions into microfluidic devices allows a platform shift from conventional cell culture models to organ-specific microenvironments, offering a better model for human body response to drugs or other biological stimuli.

Lung-on-a-Chip

One area of representative pioneering work in the organ-on-a-chip field is human lung biomimetic microsystems. For instance, Huh et al. created a microdevice to model human alveolar-capillary interfaces, simulating a fundamental unit of the human lung, by bonding two microchannels separated by a thin flexible porous PDMS membrane.[5] This sandwich design enabled air flow into the upper compartment, with epithelial cells coating the membrane, and medium circulation in the lower compartment with endothelial cells coating the other side of the membrane. Vacuum application on both microchannels

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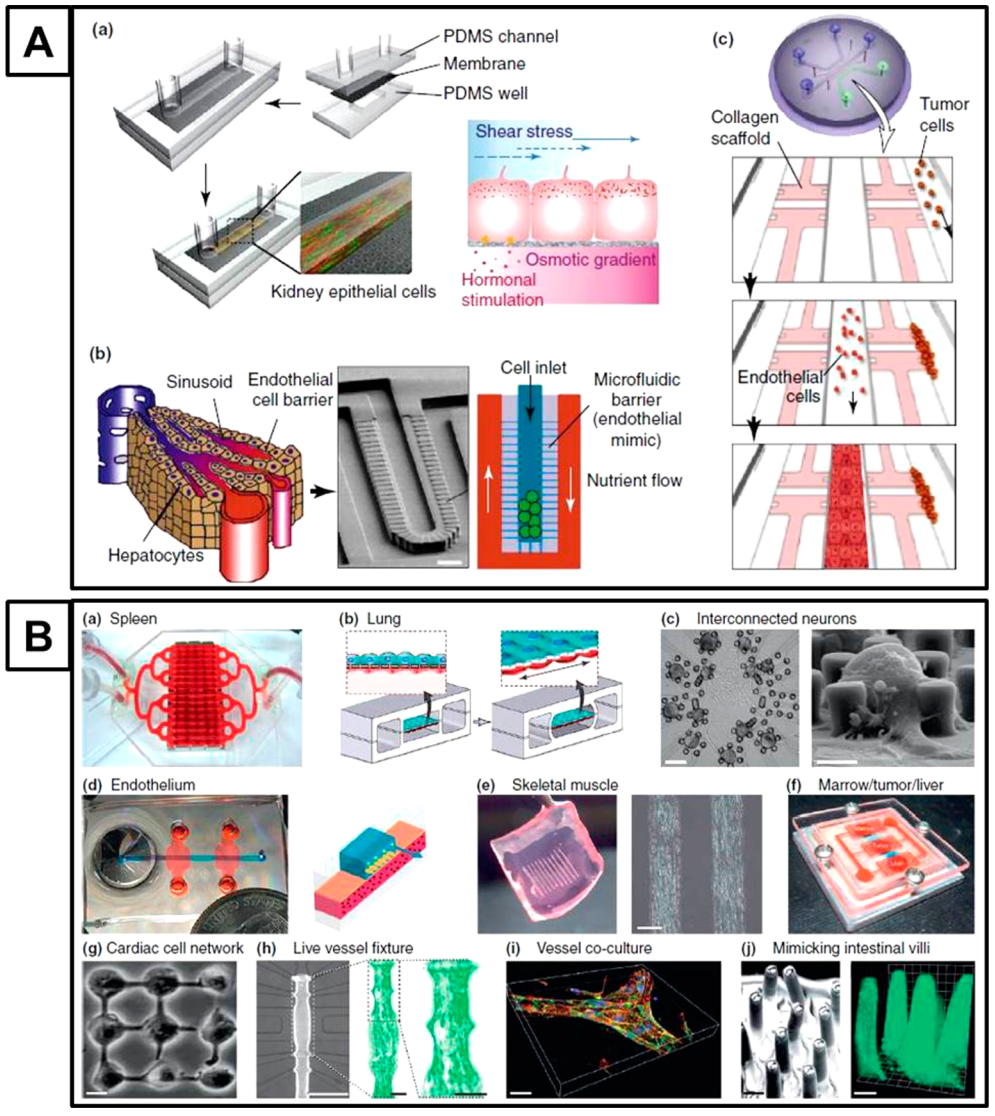

Figure 3. Organ-on-chip systems.[17,42−44] (A) The multilayer collecting-duct-on-a-chip for renal cell culture.[17] (B) Electromicrograph of (a) two-way contact with picket fence (scale bar = 20 μm), (b) of 3-day cultured neuron after fixation (scale bar = 20 μm), and (c) 2-day neuron culture in picket fences (scale bar = 100 μm).[42] (C) (a) Photograph of myotube-containing fibrin gel and (b) micrograph of myotube patterns on the gel for skeletal muscle cell assays.[43] (D) (a) PDMS villi structure scanning electron micrograph and (b) 3-D rendered collagen scaffold (confocal microscope) for human intestinal system in a microfluidic device.[44]

during cell culture facilitated growth of both cell layers at the air−liquid interface while simulating pulmonary breathing movements. Direct, real-time visualization of individual cells demonstrated that the medium containing cytokine molecules up-regulated the expression of adhesion molecules produced by endothelial cells and induced transmigration of leukocytes through confluent cell layers, whereas cyclic mechanical strain had no impact on apparent inflammatory response markers.

Liver-on-a-Chip

Hepatotoxicity is recognized as the one of the major issues that causes side effects in drug development.[13,28−30] Domansky et al. reported a reliable and efficient liver-on-a-chip platform equipped with electronically controlled pneumatic micropumps that sustained parallel 3D liver cell culture. All included tissues were maintained with constant fluidic perfusion and kept functionally viable for at least one week, facilitating high throughput observation of drug candidate hepatotoxicity.[30] Furthermore, oxygen distribution in each liver culture was modeled by a computational tool to predict gas transfer and consumption, and this model was consistent with the on-chip measurement of luminescence-based analyte concentrations.

Heart-on-a-Chip

Replicating relevant cardiac tissue is another urgent need for drug testing because of the high risk of heart failure resulting from unforeseen drug toxicities. A novel "muscular thin film" (MTF) assay has been proposed to explore the structure/function relationship and drug dose effects on anisotropic cardiac myocytes, overcoming the shortcomings of traditional single cell studies and isotropic cardiac assays.[31] The MTF device is composed of a metallic temperature controller, embedded microelectrodes, an elastomeric thin film array of cultured cardiac cells, and a transparent top to allow optical screening. PDMS thin film cantilevers and cardiac myocytes were utilized to replicate the laminar structure of the heart ventricle, and deflection of each myocyte-incorporated cantilever in the array was recorded after electrophysiological

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stimulation to calculate diastolic and systolic stresses. The utility of this MTF device was further explored by exposing the MTF-based heart-on-a-chip system to varying doses of isoprotenerol, a nonselective beta adrenergic agonist, and monitoring cardiac contractibility of the engineered tissue system. The microengineered heart-on-a-chip system showed dose-dependent changes in the cantilever deformation due to cardiac contractibility and proved its utility as a drug screening platform.

Immune-System-on-a-Chip

Rapid progress in developing organ-on-a-chip platforms has aroused interest in building pharmacokinetics-pharmacodynamics (PK-PD) models to study drug behavior in multiorgan systems.[29,40] One precursor study employed a compact three-chamber "micro cell culture analog" (μCCA) device based on a PK-PD model.[29] Sung et al. assembled this μCCA system for hydrogel cell culture of liver, tumor, and bone marrow cell lines. Gravity-generated fluid flow eliminated the need for an external pump and prevented bubble formation, and this unique flow recirculation system allowed the cells to retain viability for three days. A brief comparison of drug cytotoxicity in the theoretical PK-PD model and dynamic μCCA conditions proved that experimental results fit computational estimates satisfactorily, providing opportunities for such combined approaches to predict the drug effects in complex biological systems.

Like the innovations detailed in the previous sections, the proper adaptation or use of these on-chip mimetics of living organs will lead to better understanding of human biology. The main challenge of this newly emerging technology is that, like all other cutting-edge technologies, these devices are currently only accessible to those with the expertise and equipment to design and fabricate the devices. Significant interdisciplinary expertise and effort are required to design, fabricate, and apply these devices. Hopefully, the great efforts, like those detailed in the above examples, will bring organ-on-a-chip technology from cutting-edge to practical use in areas like drug metabolism studies, which are typically time-consuming, expensive, and often fail to translate to clinical drug tests.

■ CONCLUSIONS

As described above, microfluidic mimetics of in vivo environments are emerging as powerful platforms to study human pathophysiology. These pioneering efforts have replicated in-vivo-relevant environments on an in vitro platform and have identified critical cell−flow, cell−chemical mediator, and cell−cell interactions that influence cellular behaviors, validating the on-chip in vivo mimetics as a good model for human biology studies. Future effort in this field will allow even more nuanced modeling of human immune systems, bacterial systems, ecosystems, and even interactions among these on a microfluidic system and provide opportunities to significantly improve our understanding of human biology, and thus, public health.

Of course, despite the recent achievements described above, the field is still in need of further innovation. One critical need is easy incorporation of more detection/analysis methods. Nearly all microfluidic devices employ optical microscopy techniques (e.g., phase contrast, immunofluorescence, or selective plane illumination microscopy[41]); however, realization of drug screening or transplant assessment will require simultaneous measurement of various cellular mediators and markers that all need to be quantitatively analyzed in a spatially and temporally resolved manner. There have been significant research efforts to enable state-of-the-art detection on microfluidic platforms: surface-enhanced Raman scattering, mass spectrometry, NMR, electrophoresis, and electrochemistry have all been recently reported.[6−8,32] There has also been intensive research effort to incorporate conventional biochemical assays, such as the polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), onto microfluidic platforms.[33]

Another challenge that on-chip in vivo mimetic systems, as well as the entire field of human biology, faces is the lack of available human cells and appropriate culture media. Even the available human cells are not sufficiently stable for long-term in vitro cultures, making multiple organ-combined systems like the immune system on-a-chip very difficult. In addition, different cell types often thrive in distinct culture media, making it difficult to coculture cell types while maintaining optimum behavior from each type. One promising alternative is the use of stem cells; however, further development of stem cells is deterred by ethical concerns.

Lastly, microfluidic devices will require more user-friendly interfaces to be employed as point-of-care diagnostics systems or analytical platforms for fundamental sciences. This requires, in addition to reproducibility and reliability as an analytical platform, easy operation by unskilled users. Clearly, this requires simplification of fabrication protocols, achieving mass production of such in vivo-mimetic systems, and automation of device operation.

The above innovations will require significant input from materials chemists, analytical chemists, cellular biologists, pharmacologists, and engineers. Such developments in the field will significantly benefit our fundamental understanding of human pathophysiology and improve current pharmaceutical and medical applications.

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REFERENCES

(1) Huh, D.; Torisawa, Y. S.; Hamilton, G. A.; Kim, H. J.; Ingber, D. E. Microengineered Physiological Biomimicry: Organs-on-Chips. *Lab Chip* **2012**, *12*, 2156−2164.
(2) Zheng, C.; Zhao, L.; Chen, G.; Zhou, Y.; Pang, Y.; Huang, Y. Quantitative Study of the Dynamic Tumor−Endothelial Cell Interactions through an Integrated Microfluidic Coculture System. *Anal. Chem.* **2012**, *84*, 2088−2093.
(3) Kastrup, C. J.; Runyon, M. K.; Lucchetta, E. M.; Price, J. M.; Ismagilov, R. F. Using Chemistry and Microfluidics to Understand the Spatial Dynamics of Complex Biological Networks. *Acc. Chem. Res.* **2008**, *41*, 549−558.
(4) Colace, T. V.; Tormoen, G. W.; McCarty, O. J. T.; Diamond, S. L. Microfluidics and Coagulation Biology. *Annu. Rev. Biomed. Eng.* **2013**, *15*, 283−303.
(5) Huh, D.; Hamilton, G. A.; Ingber, D. E. From 3D Cell Culture to Organs-on-Chips. *Trends Cell Biol.* **2011**, *21*, 745−754.
(6) Chrimes, A. F.; Khoshmanesh, K.; Stoddart, P. R.; Mitchell, A.; Kalantar-zadeh, K. Microfluidics and Raman Spectroscopy: Current Applications and Future Challenges. *Chem. Soc. Rev.* **2013**, *42*, 5880−5906.
(7) Nge, P. N.; Rogers, C. I.; Wooley, A. T. Advances in Microfluidic Materials, Functions, Integration, and Applications. *Chem. Rev.* **2013**, *113*, 2550−2583.
(8) Lee, H.; Sun, E.; Ham, D.; Weissleder, R. Chip−NMR Biosensor for Detection and Molecular Analysis of Cells. *Nat. Med.* **2008**, *14*, 869−874.
(9) Huh, D.; Matthews, B. D.; Mammoto, A.; Montoya-Zavala, M.; Hsin, H. Y.; Ingber, D. E. Reconstituting Organ-Level Lung Functions on a Chip. *Science* **2010**, *328*, 1662−1668.
(10) Chiu, D. T.; Lorenz, R. M. Chemistry and Biology in Femtoliter and Picoliter Volume Droplets. *Acc. Chem. Res.* **2009**, *42*, 649−658.
(11) Sivagnanam, V.; Gijs, M. A. M. Exploring Living Multicellular Organisms, Organs, and Tissues using Microfluidic Systems. *Chem. Rev.* **2013**, *113*, 3214−3247.
(12) Kim, D.; Haynes, C. L. On-Chip Evaluation of Neutrophil Activation and Neutrophil−Endothelial Cell Interaction during Neutrophil Chemotaxis. *Anal. Chem.* **2013**, *85*, 10787−10796.
(13) Jang, K.-J.; Suh, K.-Y. A Multi-Layer Microfluidic Device for Efficient Culture and Analysis of Renal Tubular Cells. *Lab Chip* **2010**, *10*, 36−42.
(14) Nakao, Y.; Kimura, H.; Sakai, Y.; Fujii, T. Bile Canaliculi Formation by Aligning Rat Primary Hepatocytes in a Microfluidic Device. *Biomicrofluidics* **2011**, *5*, 022212−022217.
(15) Muthard, R. W.; Diamond, S. L. Side View Thrombosis Microfluidic Device with Controllable Wall Shear Rate and Transthrombus Pressure Gradient. *Lab Chip* **2013**, *13*, 1883−1891.
(16) Wood, D. K.; Soriano, A.; Mahadevan, L.; Higgins, J. M.; Bhatia, S. N. A Biophysical Indicator of Vaso-Occlusive Risk in Sickle Cell Disease. *Sci. Transl. Med.* **2012**, *4*, 123ra26−123ra26.
(17) Jang, K. J.; Cho, H. S.; Kang, D. H.; Bae, W. G.; Kwon, T. H.; Suh, K. Y. Fluid-Shear-Stress-Induced Translocation of Aquaporin-2 and Reorganization of Actin Cytoskeleton in Renal Tubular Epithelial Cells. *Integr. Biol.* **2011**, *3*, 134−141.
(18) Zheng, Y.; Chen, J.; Craven, M.; Choi, N. W.; Totorica, S.; Diaz-Santana, A.; Kermani, P.; Hempstead, B.; Fischbach-Teschl, C.; Lopez, J. A.; Stroock, A. D. Vitro Microvessels for the Study of Angiogenesis and Thrombosis. *Proc. Natl. Acad. Sci.* **2012**, *109*, 9342−9347.
(19) Kim, D.; Finkenstaedt-Quinn, S.; Hurley, K. R.; Buchman, J. T.; Haynes, C. L. On-Chip Evaluation of Platelet Adhesion and Aggregation upon Exposure to Mesoporous Silica Nanoparticles. *Analyst* **2014**, DOI: 10.1039/C3AN01679J.
(20) Korin, N.; Kanapathipillai, M.; Matthews, B. D.; Crescente, M.; Brill, A.; Mammoto, T.; Ghosh, K.; Jurek, S.; Bencherif, S. A.; Bhatta, D.; Coskun, A. U.; Feldman, C. L.; Wagner, D. D.; Ingber, D. E. Shear-Activated Nanotherapeutics for Drug Targetting to Obstructed Blood Vessels. *Science* **2012**, *337*, 738−742.
(21) Kim, D.; Haynes, C. L. The Role of p38 MAPK in Neutrophil Functions: Single Cell Chemotaxis and Surface Marker Expression. *Analyst* **2013**, *138*, 6826−6833.
(22) Skelley, A. M.; Kirak, O.; Suh, H.; Jaenisch, R.; Voldman, J. Microfluidic Control of Cell Pairing and Fusion. *Nat Methods* **2009**, *6*, 147−152.
(23) Ma, H. P.; Liu, T. J.; Qin, J. H.; Lin, B. C. Characterization of the Interaction between Fibroblasts and Tumor Cells on a Microfluidic Co-Culture Device. *Electrophoresis* **2010**, *31*, 1599−1605.
(24) Hsu, T. H.; Xiao, J. L.; Tsao, Y. W.; Kao, Y. L.; Huang, S. H.; Liao, W. Y.; Lee, C. H. Analysis of the Paracrine Loop between Cancer Cells and Fibroblasts using a Microfluidic Chip. *Lab Chip* **2011**, *11*, 1808−1814.
(25) Kim, J.; Hegde, M.; Jayaraman, A. Co-Culture of Epithelial Cells and Bacteria for Investigating Host−Pathogen Interactions. *Lab Chip* **2010**, *10*, 43−50.
(26) Hong, J. W.; Song, S.; Shin, J. H. A Novel Microfluidic Co-Culture System for Investigation of Bacterial Cancer Targeting. *Lab Chip* **2013**, *13*, 3033−3040.
(27) Hsiao, A. Y.; Torisawa, Y. S.; Tung, Y. C.; Sud, S.; Taichman, R. S.; Pienta, K. J.; Takayama, S. Microfluidic System for Formation of PC-3 Prostate Cancer Co-Culture Spheroids. *Biomaterials* **2009**, *30*, 3020−3027.
(28) Ghaemmaghami, A. M.; Hancock, M. J.; Harrington, H.; Kaji, H.; Khademhosseini, A. Biomimetic Tissues on a Chip for Drug Discovery. *Drug Discovery Today* **2012**, *17*, 173−181.
(29) Sung, J. H.; Kam, C.; Shuler, M. L. A Microfluidic Device for a Pharmacokinetic-Pharmacodynamic (PK-PD) Model on a Chip. *Lab Chip* **2010**, *10*, 446−455.
(30) Domansky, K.; Inman, W.; Serdy, J.; Dash, A.; Lim, M. H.; Griffith, L. G. Perfused Microwell Plate for 3D Liver Tissue Engineering. *Lab Chip* **2010**, *10*, 51−58.
(31) Agarwal, A.; Goss, J. A.; Cho, A.; McCain, M. L.; Parker, K. K. Microfluidic Heart on a Chip for Higher Throughput Pharmacological Studies. *Lab Chip* **2013**, *13*, 3599−3608.
(32) Lee, J.; Soper, S. A.; Murray, K. K. Microfluidic Chips for Mass Spectrometry-Based Proteomics. *J. Mass Spectrom.* **2009**, *44*, 579−593.
(33) Chin, C. D.; Laksanasopin, T.; Cheung, Y. K.; Steinmiller, D.; Linder, V.; Parsa, H.; Wang, J.; Moore, H.; Rouse, R.; Umviligihozo, G.; Karita, E.; Mwambarangwe, L.; Braunstein, S. L.; van de Wijgert, J.; Sahabo, R.; Justman, J. E.; El-Sadr, W.; Sia, S. K. Microfluidics-based Diagnostics of Infectious Diseases in the Developing World. *Nat. Med.* **2011**, *17*, 1015−1019.
(34) Zervantonakis, I. K.; Hughes-Alford, S. K.; Charest, J. L.; Condeelis, J. S.; Gertler, F. B.; Kamm, R. D. Three-dimensional Microfluidic Model for Tumor Cell Intravasation and Endothelial Barrier Function. *Proc. Natl. Acad. Sci.* **2012**, *109*, 13515−13520.
(35) Lee, S.-A.; No, D. Y.; Kang, E.; Ju, J.; Kim, D.-S.; Lee, S.-H. Spheroid-Based Three-Dimensional Liver-on-a-Chip to Investigate Hepatocyte−Hepatic Stellate Cell Interactions and Flow Effects. *Lab Chip* **2013**, *13*, 3529−3537.
(36) Patra, B.; Chen, Y.-H.; Peng, C.-C.; Lin, S.-C.; Lee, C.-H; Tung, Y.-C. A Microfluidic Device for Uniform-sized Cell Spheroids Formation, Culture, Harvesting and Flow Cytometry Analysis. *Biomicrofluidics* **2013**, *7*, No. 054114.
(37) Moraes, C.; Mehta, G.; Lesher-Perez, S. C.; Takayama, S. Organs-on-a-chip: A Focus on Compartmentalized Microdevices. *Ann. Biomed. Eng.* **2011**, *40*, 1211−1227.
(38) Kovarik, M. L.; Gach, P. C.; Ornoff, D. M.; Wang, Y.; Balowski, J.; Farrag, L.; Allbritton, L. Micro Total Analysis Systems for Cell Biology and Biomedical Assays. *Anal. Chem.* **2012**, *84*, 516−540.
(39) Chan, C. Y.; Huang, P.-H.; Guo, F.; Ding, X.; Kapur, V.; Mai, J. D.; Yuen, P. K.; Huang, T. J. Accelerating Drug Discovery via Organs-on-Chips. *Lab Chip* **2013**, *13*, 4697−4710.
(40) Maguire, T. J.; Novik, E.; Chao, P.; Marminko, J.; Nahmias, Y.; Yarmush, M. L.; Cheng, K.-C. Design and Application of Microfluidic

Systems for In Vitro Pharmacokinetic Evaluation of Drug Candidates. *Curr. Drug Metab.* **2009**, *10*, 1192−1199.
(41) Pampaloni, F.; Reynaud, E. G.; Stelzer, E. H. K. The Third Dimension Bridges the Gap between Cell Culture and Live Tissue. *Nat. Rev. Mol. Cell Biol* **2007**, *8*, 839−845.
(42) Zeck, G.; Fromherz, P. Noninvasive Neuroelectronic Interfacing with Synaptically Connected Snail Neurons Immobilized on a Semiconductor Chip. *Proc. Natl. Acad. Sci.* **2001**, *98*, 10457−10462.
(43) Nagamine, K.; Kawashima, T.; Sekine, S.; Ido, Y.; Kanzaki, M.; Nishizawa, M. Spatiotemporally Controlled Contraction of Micropatterned Skeletal Muscle Cells on a Hydrogel Sheet. *Lab Chip* **2011**, *11*, 513−517.
(44) Sung, J. H.; Yu, J.; Luo, D.; Shuler, M. L.; March, J. C. Microscale 3-D Hydrogel Scaffold for Biomimetic Gastrointestinal (GI) Track Model. *Lab Chip* **2011**, *11*, 389−392.