Annals of Biomedical Engineering (© 2011)
DOI: 10.1007/s10439-011-0455-6

BMES BIOMEDICAL ENGINEERING SOCIETY™ www.bmes.org

Organs-on-a-Chip: A Focus on Compartmentalized Microdevices

CHRISTOPHER MORAES,[1] GEETA MEHTA,[1] SASHA CAI LESHER-PEREZ,[1] and SHUICHI TAKAYAMA[1,2,3]

[1]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA; [2]Department of Macromolecular Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA; and [3]Division of Nano-Bio and Chemical Engineering WCU Project, UNIST, Ulsan, Republic of Korea

(*Received 22 August 2011; accepted 24 October 2011*)

Associate Editor Michael Shuler oversaw the review of this article.

Abstract—Advances in microengineering technologies have enabled a variety of insights into biomedical sciences that would not have been possible with conventional techniques. Engineering microenvironments that simulate *in vivo* organ systems may provide critical insight into the cellular basis for pathophysiologies, development, and homeostasis in various organs, while curtailing the high experimental costs and complexities associated with *in vivo* studies. In this article, we aim to survey recent attempts to extend tissue-engineered platforms toward simulating organ structure and function, and discuss the various approaches and technologies utilized in these systems. We specifically focus on microtechnologies that exploit phenomena associated with compartmentalization to create model culture systems that better represent the *in vivo* organ microenvironment.

Keywords—Organ, Compartment, Microtechnology, Microengineering, Microenvironment, Barrier.

INTRODUCTION

Utilizing animal models to study various aspects of development, homeostasis, and diseased behavior is a critical step in understanding, predicting, and eventually controlling biological function. *In vivo* experiments form a crucial bridge between carrying out experiments in a Petri dish and developing viable clinical and technological solutions for current challenges to human health and quality of life. However, *in vivo* models can be expensive, highly variable, difficult to manipulate, and experimental results can often be confounded or challenging to interpret. As a result, in the pharmaceutical industry, for example, *in vivo* studies require exorbitant resources, but often fail to translate promising *in vitro* results for drug compounds to clinically viable solutions.[103] One factor that could account for these discrepancies is the inability of the conventional *in vitro* models to capture the critical features of the cellular microenvironment, which may influence or modulate cellular response to the therapy.[34] Hence, *in vitro* culture systems of sufficient complexity may provide an alternative, low-cost, preclinical test platform with improved relevance and utility in identifying therapeutic avenues that warrant *in vivo* testing. Furthermore, such artificial surrogates would enable the precise manipulation of specific environmental parameters to tease out the underlying mechanisms for biological phenomena, and provide a greater understanding of the underlying biology.

While static Petri dish culture has been utilized for decades, it is simply unable to capture the structural, mechanical, chemical, and communicative complexities of *in vivo* systems. The development of engineered tissues as model systems has partially bridged this gap, and recent efforts have focused on making these models more relevant by creating an organ-like microenvironment. Micro-engineered strategies provide a number of unique advantages and benefits in studying organ biology. The ability to pattern relatively large surfaces with subcellular resolution features allows precise control over various aspects of the cellular microenvironment, while maintaining the size necessary to allow for complex interactions between system components. In this review, we highlight the use of microtechnologies in simulating critical features of *in vivo* organs, and specifically focus on microdevices that leverage compartmentalization strategies to move toward re-creating complex organ microenvironments that bridge the gap between *in vitro* and *in vivo* models.

Address correspondence to Shuichi Takayama, Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. Electronic mail: takayama@umich.edu

The first three authors contributed equally in this study.

Published online: 08 November 2011

FROM TISSUES TO ORGANS

Basic biology textbooks ubiquitously define a "tissue" as a group of cells that act together to perform one or more functions. Similarly, an "organ" is a group of tissues that perform a specific function or group of functions. Though students have no practical difficulties in identifying and differentiating between organs in the body, they often encounter problems in applying this definition in the classification process.[8] This may be due to the hierarchical nature of the definition, in which "tissues" and "organs" are not distinguished based on a level of functional capability, but rather on arbitrarily assigned levels of complexity. We encountered similar issues in defining inclusion criteria for this review: while tissue-like multicellular constructs are often cultured on microengineered platforms, researchers are currently unable to fully recapitulate the complete structure or function of a native organ. In order to provide a systematic review of the current state of the art toward creating organs on a chip, we have adopted the hierarchical aspect of the standard definition, and based our selection criteria on level of complexity, rather than on specific requirements of function, output, or accuracy in biological imitation.

For the purposes of this review, we define "tissues" as those systems in which (1) biological material is cultured with at least the potential for long-term studies; (2) multicellular constructs of one cell type are formed; and (3) the resulting tissue recapitulates some aspect of the native *in vivo* tissue. Following this definition, examples of a "*tissue*-on-a-chip" would include microengineered systems in which tissue constructs are formed using various techniques[106] (such as cell trapping in microfluidic devices[11,58]), under the influence of factors such as substrate topography,[39] fluid shear,[29] or perfused culture.[14] We chose to identify "*organs*-on-a-chip" as those systems that extend upon these minimum characteristics to better recapitulate the *in vivo* organ milieu, either within a distinct organ structure or between multiple organ systems. To narrow the scope of this article, we focus specifically on those microsystems that utilize microscale compartmentalization strategies to achieve this goal.

THE ROLE OF MICROENGINEERED COMPARTMENTS

Compartmentalization can broadly be described as creating separations between differentiated environments (Fig. 1). Compartmentalization can take the form of imposing constraints on the physical environment to precisely define microenvironmental conditions, and controlling the nature of the separation barrier between compartments. This approach broadly mimics organ structure and function, in which specialized tissues in well-defined environments interact with each other via several modalities to generate organ function. Using microtechnologies to define such compartments yields significant advantages in re-creating organ function. Specifically, microengineering compartments results in the ability to (1) create unique conditions arising from favorable scaling laws, (2) simultaneously and precisely control multiple chemical and physical culture conditions with a spatial resolution appropriate for cell and tissue culture, and (3) manipulate chemical and physical communication between such environments.

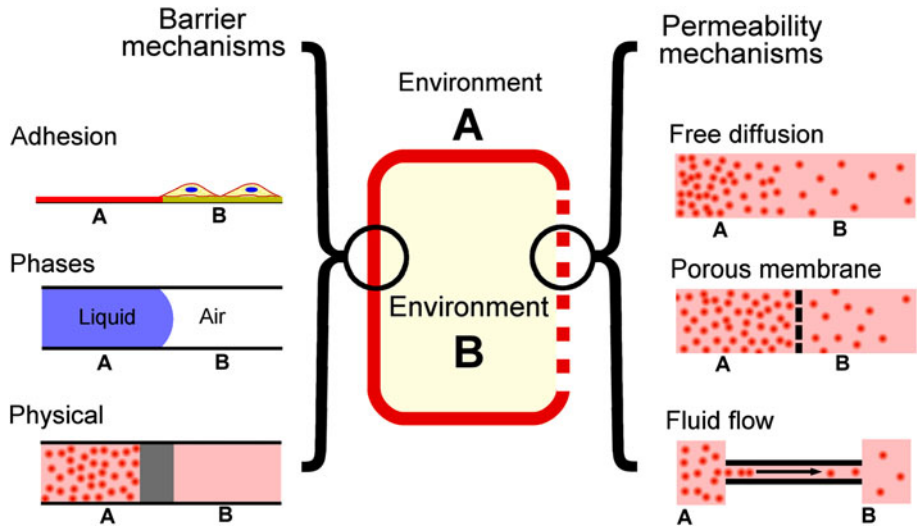

FIGURE 1. Schematic representation of compartmentalization between environments A and B, with examples of potential barrier and permeability mechanisms between environments.

First, using microtechnologies to create such compartments allows us to establish environmental conditions that would not have been possible with conventional macroscale techniques. For example, in a simple microfluidic compartment in which cells are confined within a small volume, physiological concentrations of secreted soluble signaling molecules can be achieved,[89] a critical feature in re-creating organ conditions of communication between tissue types. The confined regions also allow us to reliably create and sustain gradients of soluble molecules, a characteristic feature associated with *in vivo* organogenesis and development.[45] Laminar flow in microchannels also enables the formation of sub-compartments within the channel. Additional examples of microscale fluidic sub-compartmentalization include the creation of traveling air and liquid compartments, which can simulate diseased conditions within the lung.[33]

Second, given the interdependent nature of cell–environment interactions, experimental platforms capable of simultaneously manipulating multiple aspects of the cellular environment are required to obtain a better understanding of how cells integrate and respond to external cues within and between tissues and organs.[55,65] Compartmentalized regions designed to manipulate several environmental parameters allow researchers to precisely define multiple spatial, chemical, and physical culture cues that best represent the organ milieu. Furthermore, the number of experimental conditions necessary for a parametric study of organ culture parameters increases exponentially with the number of parameters being tested. Creating microscale compartments reduces the resource footprint of an experiment, enabling multiple replicates to be tested simultaneously. Valving structures,[21,23,42,77,91] droplet microfluidics,[90] or varied features across an array[54,56,74] enables multiple parameters to be screened in the same experiment. Hence, exploiting microscale compartments can greatly scale up our ability to rapidly probe multiple, interacting environmental parameters that are of importance to organ systems.

Third, the ability to manipulate communication and measure functional interactions between these defined environments is of critical importance in understanding how tissues work together to produce functional organs. Communication between tissues is mediated by transport of soluble molecules or physical forces, and can be manipulated by controlling mass and energy transport properties between compartments. For example, in compartments defined by walls but connected *via* a controllable liquid bridge, fluid flow can be used to control soluble signaling molecule movement between multiple culture environments.[81] Such approaches are particularly powerful in dissecting the complex feedback loops that exist within and between tissues and organs. Hence, the ability to locally position and constrain cells, control transport properties between compartments and observe the resulting functional behavior is a crucial approach in furthering our understanding of the cellular basis for organ function.

CURRENT PROGRESS TOWARD ORGANS-ON-A-CHIP

In recent years, several organ systems have been re-created *in vitro* using compartmentalized microengineering approaches. These include a kidney,[36] cartilage,[2] intestine,[40,100] and bone-on-a-chip[44,102]; and systems that stimulate pancreatic function,[72,101] among many others. We have elected to focus only on those systems which artificially recapitulate features of *in vivo* organ microenvironments, and have hence excluded those articles which focus on forming tissues, improving analytic ability, or creating non-physiologically relevant test environments. Furthermore, due to space constraints, we only highlight (in arbitrary order) those organs for which several microsystem-based methods and approaches have been published.

Pulmonary

Pulmonary systems are responsible for gas exchange between air and blood, and remain one of the most challenging systems to study *in vivo*. The geometric and compositional structure of the lung is highly complex, preventing straightforward manipulation and observation of cells at different regions of the lung. Differences between humans and animal models make it difficult to generalize results to human pathologies, and such approaches suffer from several confounding effects. In response, researchers have developed microfabricated devices to mimic lung structure and function in the small airway and alveolar regions of the lung.

The small airways conduct air between the external environment and the alveolar structures at which gas exchange occurs across the air–blood barrier of the lungs. The small airways are lined with epithelial cells, and are subject to a variety of mechanical and chemical stimuli, arising from inhaled particles, infection, and complications from various diseases. Several pathological conditions including pneumonia, pulmonary fibrosis, or bronchitis are accompanied by distinctive symptomatic crackling sounds, believed to be caused by the clearing of airway occlusions.[66] For example, in the case of fluid-filled occlusions, computational modeling suggests that propagation and rupture of

these "liquid plugs" can cause a variety of unexpectedly significant mechanical conditions.[6,19] Particularly, when the interfacial tensions are high, as can occur during surfactant dysfunction, large gradients of shear stress and pressure are caused which may have deleterious effects on cell function and viability.[82]

In order to understand the effects of liquid plug propagation and rupture, Huh *et al.* developed a microfluidic system to create propagating compartments of air and liquid over a microfluidically engineered model epithelium. Using a microfluidic liquid plug generator (Fig. 2), they delivered picoliter volumes of occluding fluid to an epithelial sheet cultured at an air–liquid interface in a microfluidic channel. This required the development of a two-compartment membrane culture system within the channel, as culture at an air–liquid interface has been shown to promote cellular differentiation toward a more *in vivo*-like epithelial sheet.[18] The need for precise control over multiple microenvironmental parameters such as small volume liquid plug formation and air–liquid interface culture makes microfluidic compartments uniquely well suited for these studies.[83] As the plugs propagate, they shed liquid, eventually causing plug rupture. The group found that liquid plug rupture caused crackling noises similar to those heard clinically, and also found that repeated rupture of plugs caused cell death. Hence, insights are obtained validating that the clinical symptoms associated with such stethoscope sounds, particularly when combined with surfactant dysfunction may exacerbate lung pathophysiology.[33] The group has gone on to show experimentally and numerically, that a clinical surfactant reduces the interfacial tension and the fluid shear and pressure gradient profiles, modulating cell-death caused by liquid plug propagation and rupture.[84]

The alveolar structure of the lungs contains significantly different environmental features as compared to the lung airways. Solid and fluid mechanical stresses continue to play a key role; Douville *et al.* have recently attempted to capture the complexities inherent in this structure. Using a microfabricated deformable membrane on which cells are cultured, the effects of moving air–liquid compartments, in combination with deformation of the underlying substrate was used to assess the impact of fluid-filled alveolar cavities (a frequent occurrence in pneumonia), in cell death (Fig. 3a).[16] Their system successfully combines both solid- and fluid-mechanical environmental factors to mimic pathophysiological conditions including those caused by ventilator-induced lung injury, and demonstrates deleterious effects of air–liquid interfaces on cell viability can occur after as few as 30 "breaths" under severe conditions.

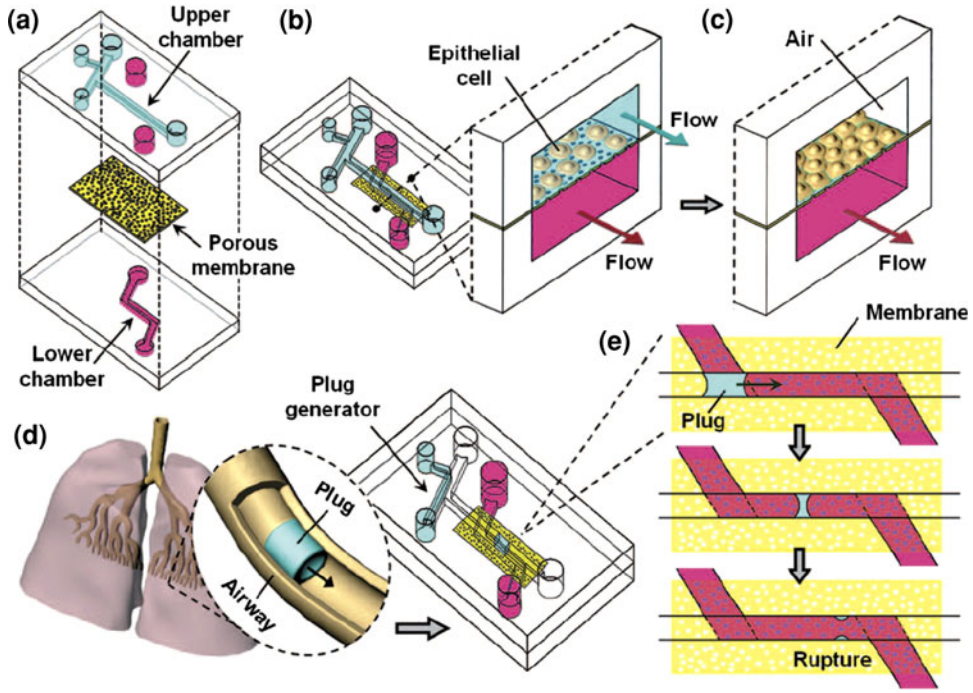

FIGURE 2. Engineered small airways on a chip (a). Takayama and co-workers developed a multilayer microdevice in which (b) epithelial cells are cultured under liquid, and (c) at an air–liquid interface. (d) A liquid plug generator is used to produce nanoliter droplets in the channel, simulating pulmonary occlusions. (e) Moving plugs shed liquid as they move, and eventually rupture, producing a crackling noise, similar to those heard in patients with pulmonary disorders. Liquid plug rupture was found to cause cell death, exacerbating lung pathophysiology (reproduced by permission from the National Academy of Sciences[33]).

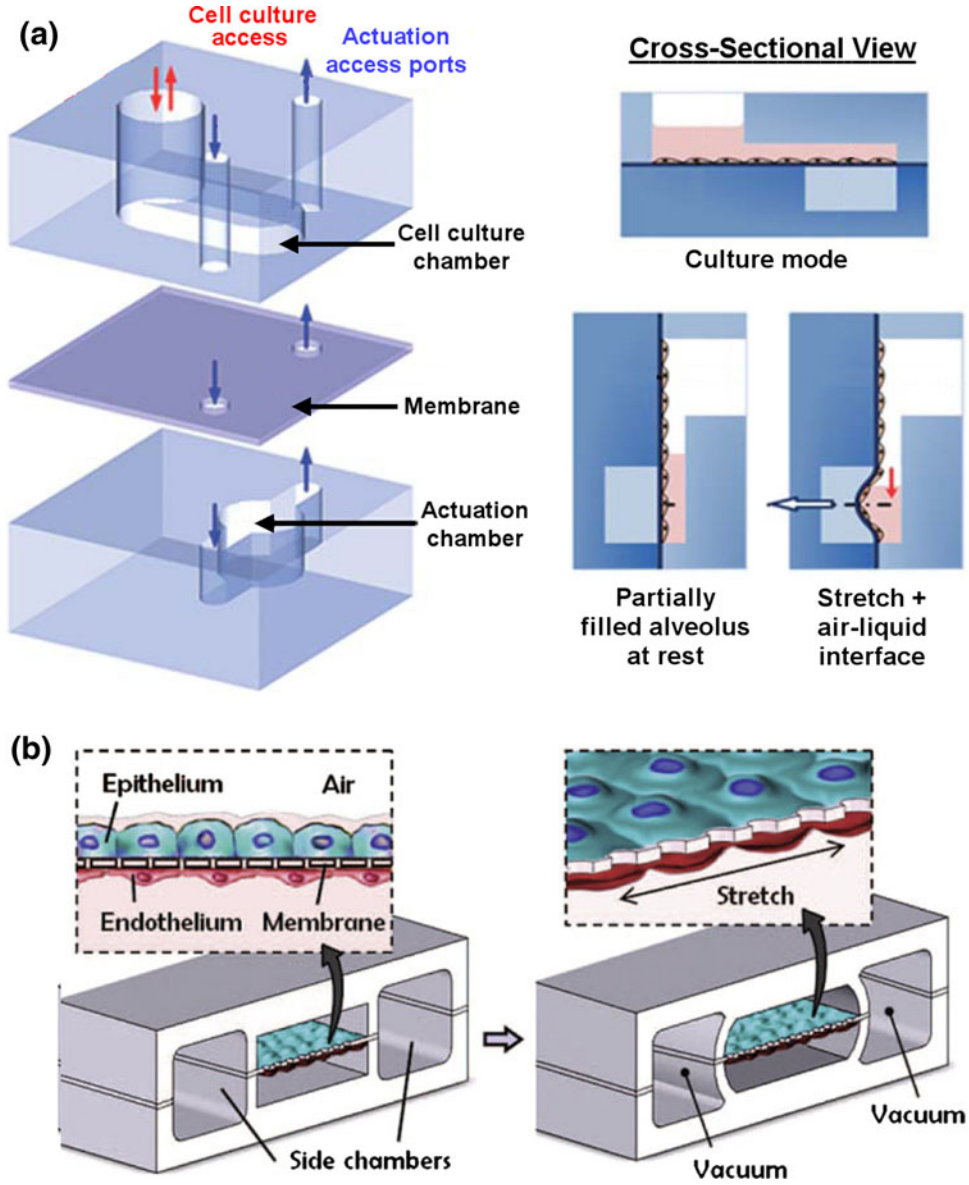

FIGURE 3. Alveoli on a chip. (a) A multilayer microfluidic device is used to culture cells on a flexible membrane, which deforms under vacuum to mechanically stress cells, while exposing them to a moving air–liquid interface similar to those found in alveolar structures under conditions of pneumonia[16] (reproduced by permission of The Royal Society of Chemistry). (b) Microdevice designed to simultaneously culture a tissue-engineered epithelium and endothelium cells, under physiologically relevant conditions of flow and stretch, to mimic conditions in the alveoli (from Huh *et al.*[34] Reprinted with permission from AAAS).

Culture at an air–liquid interface has been shown to produce an epithelium more representative of the *in vivo* state. This is difficult to perform when the cell culture substrate needs to be both flexible and permeable. For example, materials such as track-etched polycarbonate membranes are permeable,[33,57] but are stiff and do not lend themselves well to a mechanically dynamic environment. To address this, Huh *et al.* developed a porous PDMS membrane structure, integrated into an alveoli-on-a-chip device. This enabled the group to culture both alveolar epithelial cells on one side of the membrane at an air–liquid interface, and endothelial cells on the other, while applying physiologically relevant shear profiles to the endothelium and mimicking the substrate distention of the unit caused by breathing (Fig. 3b). Inflammatory responses were measured in response to simulated inhalation of nanoparticles, and these responses were mirrored in an *in vivo* murine model system. Failure to include any of the environmental stimuli resulted in a mismatch between *in vitro* and *in vivo* results.[34] This study demonstrates the utility of a microfabricated compartment with multiple, precisely defined culture parameters, to act as a surrogate for *in vivo* models, potentially

allowing for cost-effective, high-throughput screening of various drugs and treatments.

Cardiovascular

Replicating cardiovascular organs in a microsystem requires several design factors to be taken into consideration in re-creating the physiological microenvironment. Blood vessels are complex structures consisting of smooth muscle cells in the vessel walls, an endothelial layer lining the inner surface of the vessel, and a complex blood flow pattern causing fluid shear and radial deformation of the vessel[86]; some of which are ideally re-created using microtechnologies.[69,108]

Microfluidics is well adapted to applying precisely defined shear profiles to cultured cells, through careful channel design. Though syringe pumps can be used to drive flow, they can be difficult to use for generating multiple or complex flow profiles. Other approaches that allow for more multiplexed generation of flow include on-chip valves,[70,91] or commercially available Braille display devices,[77] in which periodic deformation of a channel is used to move fluid by peristaltic action. Each of these approaches have been used to drive fluid flow over a microfluidically cultured endothelium in a programmable manner, creating oscillatory and pulsatile flow profiles which better mimic the *in vivo* blood vessel microenvironment.

Closely spaced tapered microfabricated cylindrical channels within a hydrogel construct can be used as a compartmentalized model system to independently study the effects of hydrostatically driving fluid over cultured endothelial cells. Endothelial barrier function was assessed and found to depend significantly on maintaining physiological levels of both shear stress and transmural pressure.[67]

Compartmentalization within microfluidic channels can also be used to re-create other aspects of blood vessel physiology. For example, a common endothelial cell migration assay involves scratching the monolayer surface to simulate a wound. The sheet of cells then migrates to fill the gap. In order to create a more repeatable assay, researchers developed a microfluidic version of an endothelial wound. Laminar streams in a microfluidic channel are used to deliver trypsin to the central portion of a cultured endothelium. The result is a well-defined, reproducible gap in an endothelium, migration across which was then studied under physiological conditions of shear stress and stable gradients of soluble signaling molecules.[92] The study demonstrated that both physiological shear stresses and soluble molecule gradients significantly enhance wound healing, emphasizing the importance of mimicking both chemical and mechanical *in vivo* parameters in biological studies.

Complex signaling processes occur between distinct compartmentalized cell populations. Endothelial cells and smooth muscle cells cultured on either side of a porous membrane in a microfluidic device maintain separate cell populations but allow extracellular signaling. Utilizing this approach in a microfabricated platform enables endothelial cells to be regionally treated on the basal side with specific chemokines. With this method, an endothelium with differing metastatic potentials was created for a circulating breast cancer study.[76] This technique can also be used to independently apply controlled shear stress and extracellular matrix proteins to the interacting cell types, and has been used to understand the complex interactions between fluid shear and the effects of TNF-alpha on monocyte adhesion to the endothelium.[78]

Several groups have developed a series of compartmentalized microfluidic systems to study migration into a hydrogel, with excellent imaging capabilities and control over the microenvironment. One or more hydrogels integrated into a chamber between two or more adjacent microfluidic channels serves as a biologically relevant barrier.[32,95] The Kamm lab has cultured endothelial cells as a monolayer on the side-wall of the collagen gel, which are induced to migrate into the gel, under physiological conditions of interstitial fluid flow,[95] chemoattractants,[12,53] and simultaneous gradients of multiple signaling molecules.[73] The microfabricated approach provides an unprecedented ability to precisely control the microenvironment, and may be scaled up for future high-throughput studies.

Finally, a recent approach to cardiovascular studies on a chip has leveraged the ability of microfabricated systems to precisely position biological material and simultaneously manipulate the microenvironment. In their artery-on-a-chip platform, Guenther and colleagues use a microfluidic system to position an excised blood vessel within a channel, clamped in place by the application of negative pressure to specific regions. The vessel can then either be perfused or perifused with varying concentrations and gradients of biochemical solutions, to study the physiological response of the small blood vessel.[24] This hybrid approach effectively captures the physiological complexity of the biological structure, while taking advantage of the capabilities afforded by microtechnologies.

Neural

Neural system function stems from physical architecture, and from the diverse chemoelectrical and electrical properties that allow for the brain to store, access, and transmit information to control organ and tissue function. Microdevices have focused on spatio-

temporal recordings of electrophysiological signals; and have been recently utilized to engineer de novo neural systems.[26] These systems will enhance our understanding of this complex bioelectric network, and also potentially give greater insight into neural disease states.

A key microengineered approach to study neural communication and network development has been to compartmentalize cell populations from each other or cell bodies from other cells. Neuronal position and outgrowth is of critical interest in various fields from cellular biosensors to tissue engineering.[3] Surface modification is often used to separate and culture neurons while allowing neurite outgrowth through adhesive substrate patterns [68] or physical restraints such as microgrooves or channels[63] to view neuronal outgrowth ability under different conditions. Though these outgrowth systems allow for molecular and structural studies, they are unable to simulate structure of *in vivo* neural networks. However, these methods have demonstrated that compartmentalizing cells modulates neuronal communication during network development and can be applied as a tool for neural systems-on-a-chip. A compartmentalized co-culture platform was also used to promote oligodendrocyte maturation, which is critical for CNS axon myelination and improved bioelectric conductivity. By separating somatic cells from an axon/glia compartment, with an axon-guiding microchannel connecting them, oligodendrocytes were found to recapitulate *in vivo* development (Fig. 4a) allowing precursor oligodendrocytes to differentiate into mature oligodendrocytes and begin producing myelin basic protein,[63] one of the major myelin constituents.

Indirect compartmentalization has also been used in the development and analysis of electrical potential and responsiveness. Shein *et al.* used cell-adhesive carbon nanotube islands to pattern neuronal and glial cells in specific locations. These carbon nanotube islands played a double role of anchoring neurons directly to electrode sites while providing the electrical measurement resolution necessary to study the developing neural network in real time, as outgrowths occur between cells.[71] This system provided a strong proof of concept for electrical network fabrication. Another approach for cell adhesion used a surface-modifying stamping technique to constrain a neuronal network into a cell-adhesive compartment. This ring-shaped compartment aimed to reproduce persistent activity, the theoretical mechanism for fast-access memory, through a recurrently stimulating disk network. The system validated the recurrent function and persistent activity as a potential structure for such memory, by simultaneously stimulating at one location and recording from the rest of the disk network[98] (Fig. 4b).

Communication in neural tissue has been studied using compartmentalized organotypic cultures of brain slices that allow for growth and extension of axons from the cortex to the hippocampus slice. These axon extensions formed functional connections, resulting in

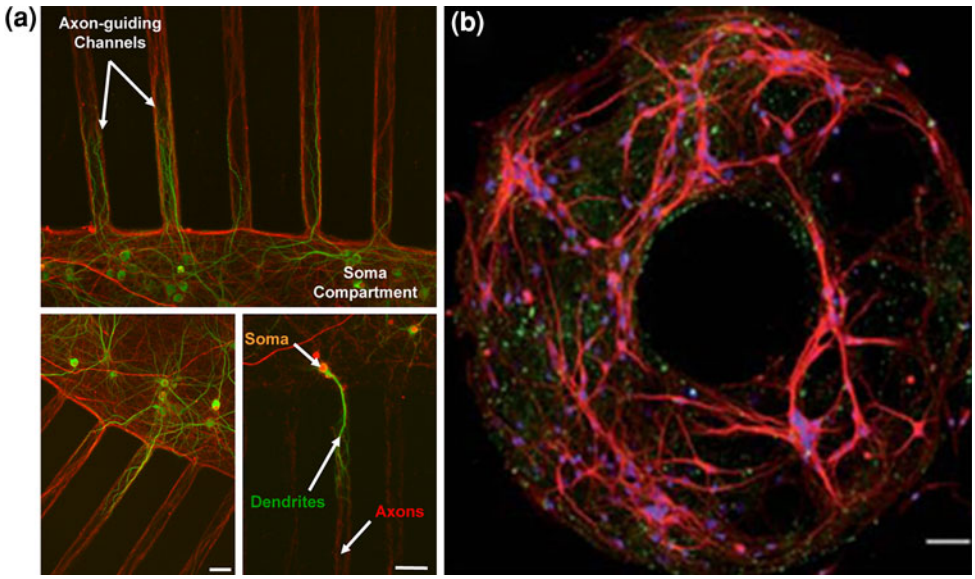

FIGURE 4. Brains on a chip. (a) Fluorescent images of neurons cultured in compartmentalized regions, with microchannel axon guides, demonstrating growth and extension away from the soma compartment toward a secondary axon-glial compartment (with kind permission from Springer Science[63]). (b) Fluorescent image of ring structure formed using microcontact printing to restrict neurons within adhesive regions, used to study recurrent networks and persistent activity (Vishwanathan *et al.*[98] reproduced by permission of The Royal Society of Chemistry).

neural activity synchronization between the slices, and provided a platform to observe pharmacological manipulation of activity in the explants. This platform enabled spatially restricted experimental manipulation of pre- and post-synaptic neurons in organotypic cultures, and can be applied to understanding the development, plasticity, and pathologies of neural pathways.[4]

Compartmentalization has been applied to specifically study soluble signaling between neural populations. Separation of primary astrocytes and microglia into microchambers has been employed in an ''overflow'' microfluidic network, allowing for controlled soluble signal communication through fluid manipulation between the two cell populations housed in their respective compartments. This overflow device demonstrated long-term co-culture and astrocyte control over purigenic receptor activation in microglia, through the well-known glutamate-induced release of ATP mechanism. This platform provides a model system to study and dissect the specific intercellular pathways involved in a variety of processes and neural disease states.[51]

Fabrication of guiding 3D structures for neural engineering has also employed compartmentalization and harnessed 3D microfluidic devices to construct neural layers and 3D architecture. Kunze *et al.*, demonstrated agarose–alginate mixtures that form multilayered scaffolds with embedded primary cortical neuron layers separated from each other by cell-free layers. B27 supplementation was delivered to form concentration gradients inducing increased neurite outgrowth. Neurite outgrowth was directed to other neurite rich regions, demonstrating the early stages of network formation within these three dimensional structures and the use of compartmentalization to promote 3D-directed outgrowth.[41] Though this method shows the potential of forming more *in vivo* like neural tissue and networks, there is still a need to further develop the system to provide an enhanced functionality from these mimetic tissue cultures.

Liver

The liver has many vital functions such as production of biomolecules, metabolization, and detoxification of ingested substances, synthesis and breakdown of small and complex molecules, decomposition of red blood cells, and filtration of impurities from the blood. The structure of the liver is complex,[59] consisting of sinusoids, or vascular channels lined with a permeable endothelium and surrounded by polarized parenchymal epithelial hepatocytes, as well as mesenchymal cells, such as macrophages, stellate cells, and lymphocytes.[9] Using compartment-based microtechnology, various research groups are working on establishing liver-on-chip systems, which are advantageous for understanding liver regeneration and for high throughput drug screening, toxicity, and metabolism studies.

Co-Cultured Hepatic Sinusoids

Hepatic sinusoid models have been created by compartmentalizing hepatocytes and non-parenchymal cells such as fibroblasts into domains that allow paracrine crosstalk. Kane *et al.* micropatterned murine fibroblasts around primary rat hepatocytes with murine fibroblasts in micropatterns. Under continuous perfusion and co-culture, the hepatocytes remained metabolically functional, as evidenced by the steady production of critical metabolites.[37] In this example, physically localizing the two cell types while maintaining paracrine communication were critical in maintaining hepatocyte function, which are typically reduced in 2D culture. In a separate study, the same cells were co-cultured in microwells layered on collagen-coated plates in 500-μm islands of primary rat or human hepatocytes surrounded by fibroblasts. The differentiated functions of hepatocytes were then evaluated by several measures including toxicity of model hepatotoxins. Though significantly fewer hepatocytes were used in this microwell model system, phenotypic liver functions were maintained for several weeks longer than conventional cultures in similar multi-well formats,[38] emphasizing the importance of communications established between the two cell types. These micropatterned co-cultures are compatible with *in situ* microscopy, robotic fluid handling, as well as, plate-reader assays, making them useful for ADME/Tox (absorption, distribution, metabolism, excretion, and toxicity) screening in industrial settings. Cho *et al.*[10] took a similar approach and patterned fibroblasts either around or as a feeder layer beneath hepatocyte islands. Increased heterotypic interaction in the latter case supported higher levels of liver-specific functions and active glycogen synthesis. Hence, the ability to pattern and position multiple cell types on the microscale, which modulates heterotypic and paracrine interactions, is an essential approach to recapitulating liver function *in vitro*.

Endothelial-Like Barrier Hepatic Sinusoids

In the liver, sinusoidal endothelial cells facilitate transport of blood and plasma to the hepatic cords. To recapitulate these transport phenomena, an artificial microfluidic liver sinusoid was created by packing a high density of hepatocytes in a microchannel (Fig. 5). The hepatocytes were fed with nutrient supply *via* thin (1–2 μm thick) channels that formed the high fluidic

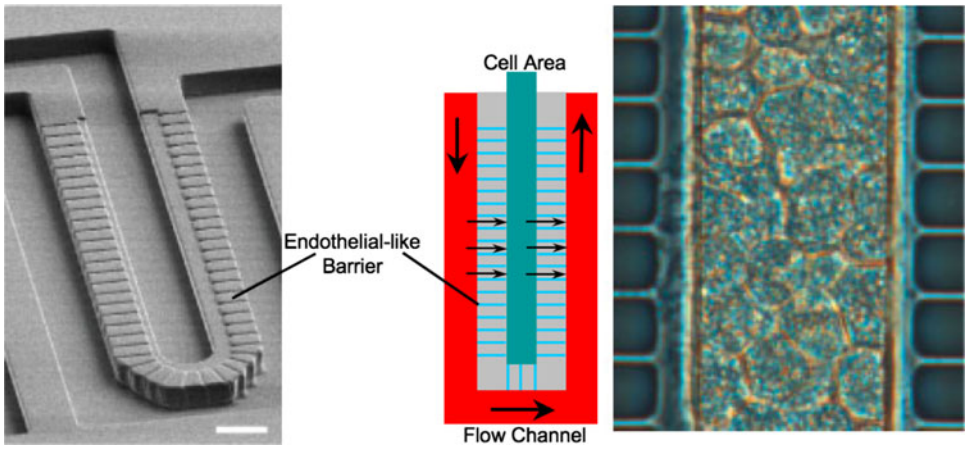

FIGURE 5. Artificial liver sinusoids on chip. *In vivo*, the sinusoid space is bordered by a sheet of highly fenestrated endothelial cells, and this highly permeable barrier enables hepatocytes to be bathed in blood plasma, while shielding them from high shear stresses. This condition is recapitulated in a simple microfluidic device in which an endothelial-like barrier of micropillars separates hepatocytes from nutrient flow. This barrier served two functions: concentrating hepatocytes in the culture area, and minimizing convective flow while allowing diffusive transport (Scale bar 20 μm). Reprinted with permission from John Wiley and Sons.[59]

resistance endothelial-like barrier between hepatocytes and the continuous convection microchannel.[46] In these artificial sinusoids, primary rat and human hepatocytes were maintained for over 7 days without losing viability, in an environment where mass transport properties of the sinusoid, such as continuous nutrient exchange and extensive cell–cell contact, were preserved. Compartmentalization of the hepatocytes and the vascular elements not only mimicked the transport processes in the sinusoid but also shielded hepatocytes from high shear stresses, known to be injurious to hepatocytes.

Perfused 3D Hepatic Cultures

It is increasingly clear that certain cells cultured in 3D are much more physiologically and clinically relevant than their counterparts in 2D.[13] To re-create the complex 3D liver microenvironment, a hepatic perfusion model was created by culturing cells within an array of micropillars and forming a thin layer of matrix over the cells.[88] Using this approach, rat hepatocytes, bone marrow mesenchymal stem cells, and human hepatoma cells were perfused for up to 1 week in compartments surrounded by micropillars, preserving their 3D morphology and cell-specific functions. This design was extended to create parallel cell culture channels independently addressed by a concentration gradient generator.[87] This 3D perfusion culture system was found to maintain functional hepatocytes at a higher level than multi-well plates, and can be readily integrated with other microdevices for the next generation of *in vitro* drug testing. Similarly, Goral *et al.*[22] used a retention micropillar array around the cell culture chamber, along with microstructures at the bottom of the chamber, which served to control the polarity of hepatocytes by minimizing cell–surface interactions and cell spreading.

Compartmentalization of human hepatocytes within the device in the absence of any exogenous matrix induced extensive 3D cell–cell interactions, leading to high viability over 2 weeks, restored gap junctions, and formation of extended bile canalicular networks. Since hepatocytes do not directly contact the perfusing medium, such devices reduce the shear stress experienced by the hepatocyte compartment by shielding them from direct flow. To address the cellular complexity of the liver sinusoids, the Griffith lab designed 3D-perfused hepatic co-cultures where cells were supported on arrays of scaffolds that were continuously perfused by pneumatic diaphragm micropumps in a multiwell plate format (Fig. 6).[35] Co-cultures of hepatocytes with stellate, Kupfer, and liver sinusoidal endothelial cells (LSEC) were successfully established, and these cells formed multicellular constructs under perfusion. Primary LSEC, which are typically difficult to culture in 2D, retained their differentiation markers, and oxygen uptake rates. Although the hepatocytes were not sheltered from shear stress in this model, it is one of the most comprehensive depictions of the diverse cell types that make up the liver, and has applications in drug toxicity and metabolism studies. The use of microfluidics enabled localized perfusion and 3D mechanical support of the tissue structure and was critical in promoting paracrine signaling between the heterogeneous cells that resulted in network-like structures representative of liver sinusoids.

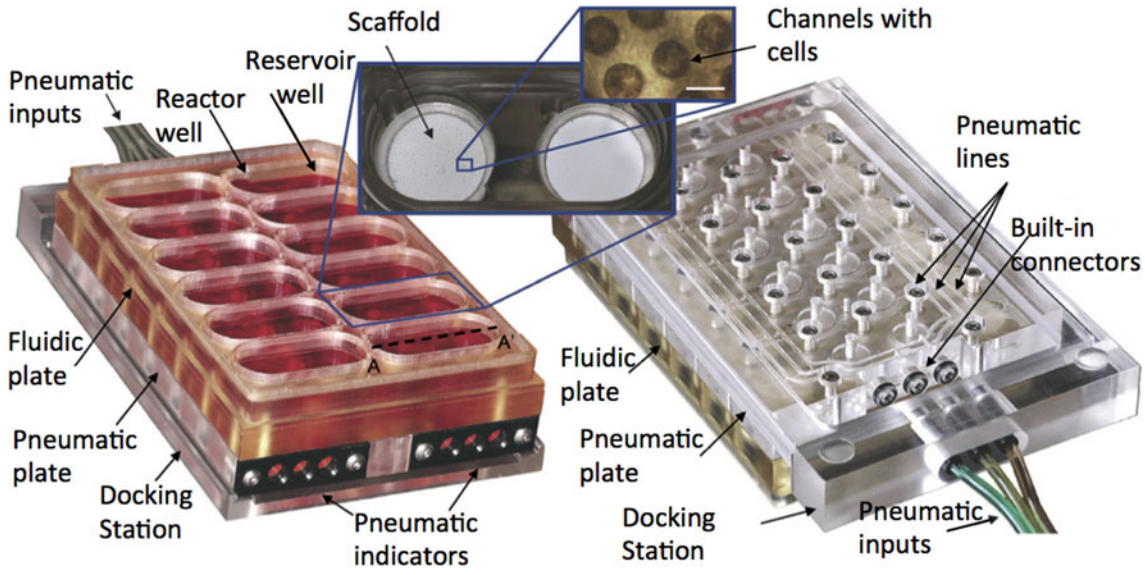

FIGURE 6. Microengineered 3-D perfused hepatic cultures on chip. Perfused multiwell bioreactor containing 12 fluidically isolated bioreactors for co-culturing hepatocytes, liver sinusoidal endothelial, stellate, and Kupfer cells (Domansky *et al.*[15]—reproduced with permission from The Royal Society of Chemistry). Perfusion through the cell-seeded scaffolds allows critical oxygen delivery immediately after seeding, and lead to formation of 3D multicellular aggregates around the scaffold pores.

Indirect Hepatocyte-Vascular Models

Given that hepatocytes have limited viability in high shear stress environments,[85] the Neville lab designed hepatic devices based on compartmentalization that allow indirect contact between hepatocytes and flowing medium through a semi-permeable membrane to limit the shear stress experienced by hepatocytes.[7,30] The sustained viability of hepatoma cells and primary rat hepatocytes under perfusion was tested in a microfluidic device with a bilayer design, comprising a fluid flow compartment and a parenchymal hepatocyte compartment separated by a semi-permeable nanoporous membrane.[7] Compared to static 2D dishes, primary rat hepatocytes expressed significantly improved markers of hepatocyte differentiation. In a separate study, these devices were seeded with rat hepatocytes and human hepatoma cells, and tested in a rodent femoral arteriovenous shunt model, where survival of liver cells was highly dependent on hepatocyte chamber pressures.[30]

Hepatocytes were protected from the high shear stresses caused by the volumetric flow rates necessary for adequate oxygen delivery by designing microgrooves in a stacked substrate, radial flow bioreactor.[64] The hepatocytes and fibroblasts co-cultured in these bioreactors maintained stable rates of synthesis of albumin and urea over 5 days. These examples illustrate the utility of using elegantly designed, simple microbioreactors to establish conditions of low-level shear stress *via* compartmentalization, required to re-create liver sinusoids.

Inter-Organ Communication

In addition to the liver, the intestine also plays a role in drug metabolism. To study the interactions between liver and intestine in the context of metabolism of hepatotoxic compounds and the regulation of bile acid homeostasis, rat intestinal and liver slices were perifused by placing a semi-permeable membrane around the organ explants, in adjoining chambers of a microfluidic device.[93] The metabolites formed by the intestine in the first chamber are passed to the liver in the second chamber for further metabolism, mimicking *in vivo* metabolism.

In another effort to study the communication between two organs in a microfluidic device, thick explants of chick embryonic liver and kidney in different compartments were cultured up to 14 days in the presence or the absence of fibronectin-coated substrates. Fibronectin was found to control migration direction between kidney and liver cells.[43] Perfusion along the microchannels facilitated migration of the organ explants toward each other, which was absent on static 2D surfaces.

Technological strides in MEMS, biomaterials, and tissue engineering provide opportunities to transform standard cell culture methods into more complex systems that retain liver function over time. As a result, more physiological models of hepatic sinusoids are becoming available, creating more predictive and reliable tools for understanding drug toxicity and disposition.

Cancer

Tumors are a heterogeneous amalgam of cells that develop through two mechanisms. The first, individual cell mutations resulting in cancer progression can be studied with conventional biochemical tools. The second, modulation of the tumor microenvironment is more challenging to study *in vitro*, but is of critical importance, as it provides spatiotemporal cues and support, necessary to progress from a few cells to malignant phenotypes. The role that non-malignant components play on the development and progression of carcinomas[5] has only recently been recognized. Carcinomas modify the stroma through expression of growth factors that promote angiogenesis, alter ECM expression, accelerate fibroblast and inflammatory cell proliferation and recruitment.[5] This dynamic interplay results in the stroma supporting growth and development of the carcinomas. Microfluidic platforms have been able to compartmentalize and study size- and spatiotemporal-dependent effects that cannot be achieved by other means.[79]

Fibroblasts are influenced by soluble factors secreted by tumor cells, which promote migration and expansive fibroblastic growth. Simple microfluidic devices have been developed to investigate cancer-driven fibroblastic responses. Ma *et al.* developed such a device and demonstrated fibroblastic affinity for different cancer cell types. This system enables multiple cell types to be cultured in connected microchambers that allowed diffusive signaling between cell types and migration between chambers. The study demonstrated a robust assay re-creating fibroblastic preference for carcinoma cells compared to an epithelial cell line (Fig. 7a).[52] This proof of concept emphasizes the necessity for stromal cell recruitment. Further study in re-creating cancer-stromal communication includes studying cancer interactions with inflammatory or endothelial cells.[20,32] Microfluidic signaling devices re-creating the tumor microenvironment have also added more realistic environmental features such as oxygen gradients[94] and biopolymer matrices and have studied more specific and complex interactions. Pneumatic valves have been used to manipulate communication between separated cell populations (Fig. 7b). The ability to use microfluidics to control the signaling waveform has been useful in analyzing paracrine loop signaling between lung cancer cells and respective fibroblasts.[31]

A similar pneumatic system was developed to dynamically modulate cross-talk between two adjacent endothelium and tumor cell populations—a critical factor in tumor growth, progression, and eventual

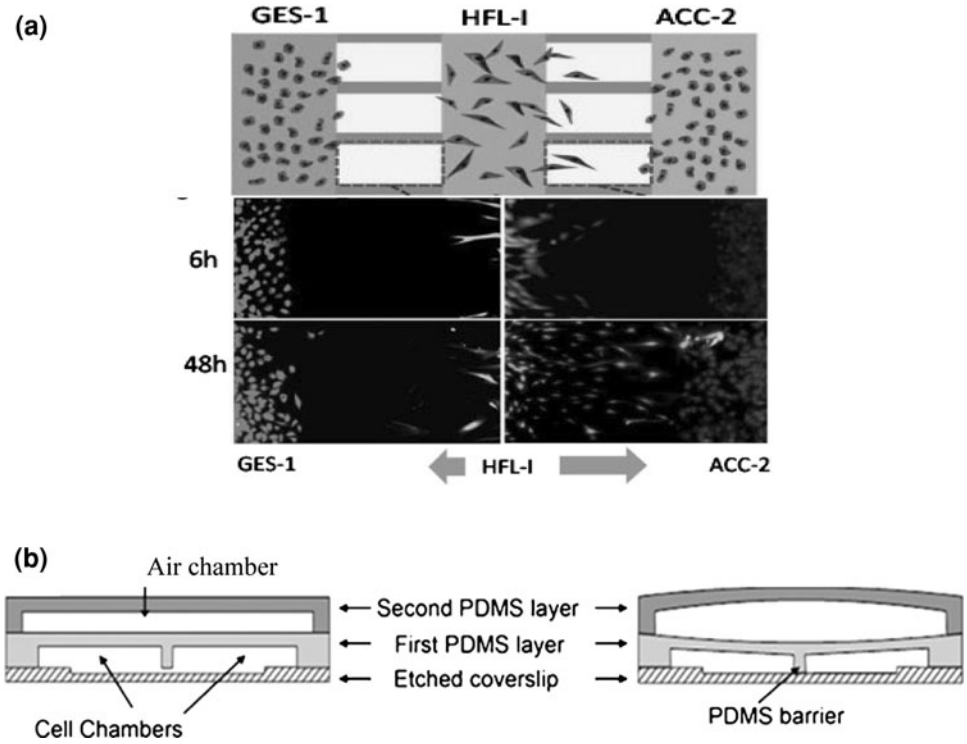

FIGURE 7. Cancer models on a chip. (a) Schematic representation and experimental images of fibroblast migration toward tumor cells (reproduced from Ma *et al.*[52] with permission from Springer); (b) PDMS-based pneumatic valve used to isolate cells into separate chambers, through the use of air pressure applied to the air chamber (reproduced from Gao *et al.*[20] with permission from John Wiley and Sons).

metastasis. This platform enabled bidirectional cell migration studies between the cellular compartments when both cell groups were sufficiently perfused with nutrients. This bidirectionality is characteristic of tumor angiogenic potential and signaling, as well as the intravasation and extravasation process of cancer cells.[20] However, microtumor niches progressing toward malignant growth create a hypoxic environment requiring oxygen delivery *via* vascular components. This study went on to induce hypoxic stress in the cancer cell compartment in the same device before allowing soluble communication between the two cell types. Induction of a hypoxic state resulted in one-way migration of endothelial cells toward tumor cells. These results mirror hypoxic tumor conditions, in which the release of angiogenic growth factors leads to migratory endothelial cell recruitment.

The stroma impacts cancer cells, promoting proliferation, migration, and metastatic potential. Torisawa *et al*. used a hydrodynamic compartmentalization system to separate cells known to be sources and sinks for stromal-derived factor 1 (SDF-1). The separated cell types created a cell-derived physiologically realistic SDF-1 gradient that caused migration in CXCR4-overexpressing breast cancer cells. This system leveraged small volumes in microchannels to produce auto/paracrine-rich microenvironments[89] and region-specific functional groups to drive gradient formation. Other studies of the interaction between fibroblasts and carcinoma cells have utilized 3D culture systems to analyze paracrine-signaling pathways and screen for inhibitors, or study the migratory response of carcinoma cells to fibroblastic regions. Liu *et al*.[50] showed communication *via* medium diffused in matrix between adjacent microchambers containing adenoid cystic carcinoma and cancer-associated fibroblasts (CAF), resulting in invasion of cancer cells into CAF-rich matrix as spheroids.

Utilizing 3D culture systems may lead to better re-creation of tumor tissue phenotypes and metabolic traits. Multicellular tumor spheroids (MCTS) simulate avascular tissue microenvironments and are accepted as tumor models in cancer research.[25,28] Microfluidic technologies have been used to robustly produce large arrays of homogenous MCTS and use them as drug-testing platforms. These microdevices employ physical barriers to separate groups of cells from each other, thereby promoting spheroid formation. Microfluidic systems are then able to dynamically perfuse the compartmentalized spheroids, allowing for improved nutrient and waste transport, similar to a tumor adjacent to capillaries *in vivo*.[1,105] To achieve better simulation of this situation, Walsh *et al*. developed a system to incorporate soluble signaling gradients present in perfused tumors. They characterized growth and diversity of cell states under these physiologically relevant conditions.[99] Avascular spheroids formed in microdevices are themselves compartmentalized into perfused and necrotic tissue portions,[1] mimicking *in vivo* cancer tumors. In addition to re-creating these structures, microfluidic systems have also been used to re-create secondary tumor sites typical of metastasis. Hsiao *et al*., reported microfluidic 3D metastatic prostate cancer model that include bone microenvironmental cues as well as metastatic prostate cancer cells. This study demonstrated physiologically representative growth rates in the co-cultured microfluidic niche.[29] Recapitulating different aspects of the tumor microenvironment provides a realistic model for pharmacological screening of new therapies, as well as a reductionist platform to study the effects of tumor developmental cues.

Animal-on-a-Chip

The ultimate extension of multi-compartment organ mimics is a system that combines multiple organ compartments together to construct an animal- or human-on-a-chip. In an effort to move toward models of the body, which account for such systemic interaction between different organs that occur naturally *in vivo*, researchers have included multiple cell types in microfluidic chips. Li and co-workers developed the integrated discrete multiple organ cell culture (IdMOC) system, in which various cells and tissue explants are cultured in wells, and allowed to communicate *via* soluble signals through an overlying layer of cell culture media.[47–49] As these systems do not leverage microengineering approaches, they are not reviewed in detail here. Simultaneously however, the Shuler group pioneered microfabricated multi-culture systems in which fluid flow is controlled between distinct organ compartments. Here, ''cell culture analog'' microbioreactors with arrays of interconnected compartments that simulate separate organ systems; each compartment having cells specific to different organs cultured in them.[17,75,80,81,96,97]

The goal of these systems was to create a physical replica of pharmacokinetic models used to assess toxicity and metabolism of drugs and chemicals. These animal-on-chip models contained chambers for culturing cells from lung, liver, tumor, and bone marrow cells that can be encapsulated in hydrogels (Fig. 8). Fluid flow between the organ chambers, circulation of metabolites and times of contacts of diluted molecules with the cells simulate *in vivo* models. Oxygen sensors integrated within the chip measure real-time metabolism.[75] Toxicity of liver toxins were modeled and evaluated in the three organs in these microcell culture analog systems.[80,81,97]

Using an approach similar to Toh *et al*.,[87] Zhang *et al*.[107] created 3D microfluidic cell culture system

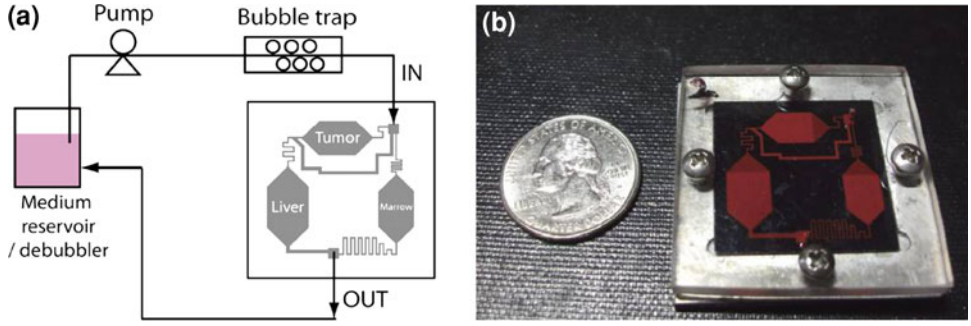

FIGURE 8. Animal on a Chip. Shuler and co-workers demonstrated the first animal-on-a-chip model, in which cells representative of certain organs are cultured in compartments which communicate *via* microfluidic channels. (a) Schematic representation and (b) experimental setup for a system in which the liver, bone marrow, and a tumor are allowed to interact (Sung and Shuler[81]—reproduced with permission from The Royal Society of Chemistry).

with compartmentalized chambers for a human-on-a-chip model. To mimic the liver, lung, kidney and the adipose tissues, four representative cell types were cultured in separate chambers, and allowed to communicate *via* soluble signals. Such animal-on-chip systems that are a first step toward realistic artificial models of interactions between physiologically complex organ chambers can be used for fundamental and applied research.

CONCLUSIONS AND FUTURE DIRECTIONS

Progress toward the development of artificial surrogates for fundamental and applied studies of biological organs has come about as a result of utilizing microtechnologies to achieve conditions not possible with conventional techniques. The emerging technologies reviewed in this article have improved our understanding of cellular behavior within the larger context of function arising from interactions between multicellular tissues. However, much study remains to be done to completely recapitulate organ function, and to progress toward human-on-a-chip model systems with greater relevance for drug discovery, and developmental and pathological studies. Furthermore, the practical utility and widespread adoption of such microengineered tools often raises concerns, in terms of both the ability of a non-specialist to operate the devices, and compatibility of the devices with existing biological assays. Though typical PDMS-and-glass microfabricated devices offer excellent conditions for optical analysis through immunohistochemistry and fluorescent microscopy, other robust and well-validated molecular biology assays such as western blots and PCR require significantly large populations of cells, which can often be difficult to obtain from microfluidic culture systems. Moreover, such assays require retrieval of the biological material from the microdevice, a feature that is often not considered in designing organs-on-a-chip. The development and integration of highly sensitive microfluidic PCR,[60] western blot assays,[27,61,62] and flow cytometry[104] systems into microdevices may alleviate these constraints, but much study remains to be done before these microfluidic modules can be considered as readily available and accessible to researchers engaged in this field. Hence, the practical utility of such microengineered platforms can often be limited in obtaining biological information, at sufficient throughput for robust hypothesis testing.

The ongoing development of improved microtechnologies to mimic cues in the organ microenvironment and to integrate with conventional biological assays will accelerate the progress toward creating human-on-a-chip model systems that are accessible, inexpensive, and readily manipulatable. Such systems will enable rapid and definitive testing of both fundamental and applied biological hypotheses. The feasibility and utility of such approaches as replacements for *in vivo* models have already been demonstrated in some cases, and further technological advances will put us a step closer toward creating a fully integrated human model.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1]Agastin, S., U. B. Giang, Y. Geng, L. A. Delouise, and M. R. King. Continuously perfused microbubble array for 3D tumor spheroid model. *Biomicrofluidics* 5(2):24110, 2011.

[2]Albrecht, D. R., G. H. Underhill, T. B. Wassermann, R. L. Sah, and S. N. Bhatia. Probing the role of multicellular organization in three-dimensional microenvironments. *Nat. Methods* 3(5):369–375, 2006.

[3]Andersson, H., and A. van den Berg. Microfabrication and microfluidics for tissue engineering: state of the art and future opportunities. *Lab Chip* 4(2):98–103, 2004.
[4]Berdichevsky, Y., K. J. Staley, and M. L. Yarmush. Building and manipulating neural pathways with microfluidics. *Lab Chip* 10(8):999–1004, 2010.
[5]Bhowmick, N. A., E. G. Neilson, and H. L. Moses. Stromal fibroblasts in cancer initiation and progression. *Nature* 432(7015):332–337, 2004.
[6]Bilek, A. M., K. C. Dee, and D. P. Gaver, 3rd. Mechanisms of surface-tension-induced epithelial cell damage in a model of pulmonary airway reopening. *J. Appl. Physiol.* 94(2):770–783, 2003.
[7]Carraro, A., W. M. Hsu, K. M. Kulig, W. S. Cheung, M. L. Miller, E. J. Weinberg, E. F. Swart, M. Kaazempur-Mofrad, J. T. Borenstein, J. P. Vacanti, and C. Neville. In vitro analysis of a hepatic device with intrinsic microvascular-based channels. *Biomed. Microdevices* 10(6):795–805, 2008.
[8]Cherif, A. H., D. M. Jedlicka, A. Al-Arabi, R. Aron, and S. Verma. Effective understanding of the human body organs: a role-playing activity for deep learning. *Am. Biol. Teach.* 72(7):447–450, 2010.
[9]Chisari, F. V., N. Fausto, D. Schachter, D. A. Shafritz, I. M. Arias, and J. L. Boyer. The Liver: Biology and Pathobiology (4th ed.). Philadelphia: Lippincott Williams & Wilkins, 2001.
[10]Cho, C. H., J. Park, A. W. Tilles, F. Berthiaume, M. Toner, and M. L. Yarmush. Layered patterning of hepatocytes in co-culture systems using microfabricated stencils. *Biotechniques* 48(1):47–52, 2010.
[11]Choudhury, D., X. Mo, C. Iliescu, L. L. Tan, W. H. Tong, and H. Yu. Exploitation of physical and chemical constraints for three-dimensional microtissue construction in microfluidics. *Biomicrofluidics* 5(2):22203, 2011.
[12]Chung, S., R. Sudo, P. J. Mack, C. R. Wan, V. Vickerman, and R. D. Kamm. Cell migration into scaffolds under co-culture conditions in a microfluidic platform. *Lab Chip* 9(2):269–275, 2009.
[13]Cukierman, E., R. Pankov, D. R. Stevens, and K. M. Yamada. Taking cell–matrix adhesions to the third dimension. *Science* 294(5547):1708–1712, 2001.
[14]Cullen, D. K., J. Vukasinovic, A. Glezer, and M. C. Laplaca. Microfluidic engineered high cell density three-dimensional neural cultures. *J. Neural Eng.* 4(2):159–172, 2007.
[15]Domansky, K., W. Inman, J. Serdy, A. Dash, M. H. Lim, and L. G. Griffith. Perfused multiwell plate for 3D liver tissue engineering. *Lab Chip* 10(1):51–58, 2010.
[16]Douville, N. J., P. Zamankhan, Y. C. Tung, R. Li, B. L. Vaughan, C. F. Tai, J. White, P. J. Christensen, J. B. Grotberg, and S. Takayama. Combination of fluid and solid mechanical stresses contribute to cell death and detachment in a microfluidic alveolar model. *Lab Chip* 11(4):609–619, 2011.
[17]Esch, M. B., T. L. King, and M. L. Shuler. The role of body-on-a-chip devices in drug and toxicity studies. *Annu. Rev. Biomed. Eng.* 13:55–72, 2011.
[18]Fritsche, C. S., O. Simsch, E. J. Weinberg, B. Orrick, C. Stamm, M. R. Kaazempur-Mofrad, J. T. Borenstein, R. Hetzer, and J. P. Vacanti. Pulmonary tissue engineering using dual-compartment polymer scaffolds with integrated vascular tree. *Int. J. Artif. Organs* 32(10):701–710, 2009.
[19]Fujioka, H., S. Takayama, and J. B. Grotberg. Unsteady propagation of a liquid plug in a liquid-lined straight tube. *Phys Fluids (1994)* 20(6):62104, 2008.
[20]Gao, Y., D. Majumdar, B. Jovanovic, C. Shaifer, P. C. Lin, A. Zijlstra, D. J. Webb, and D. Li. A versatile valve-enabled microfluidic cell co-culture platform and demonstration of its applications to neurobiology and cancer biology. *Biomed. Microdevices* 13(3):539–548, 2011.
[21]Gomez-Sjoberg, R., A. A. Leyrat, D. M. Pirone, C. S. Chen, and S. R. Quake. Versatile, fully automated, microfluidic cell culture system. *Anal. Chem.* 79(22):8557–8563, 2007.
[22]Goral, V. N., Y. C. Hsieh, O. N. Petzold, J. S. Clark, P. K. Yuen, and R. A. Faris. Perfusion-based microfluidic device for three-dimensional dynamic primary human hepatocyte cell culture in the absence of biological or synthetic matrices or coagulants. *Lab Chip* 10(24):3380–3386, 2010.
[23]Grover, W. H., A. M. Skelley, C. N. Liu, E. T. Lagally, and R. A. Mathies. Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices. *Sensors Actuators B* 89(3):315–323, 2003.
[24]Gunther, A., S. Yasotharan, A. Vagaon, C. Lochovsky, S. Pinto, J. Yang, C. Lau, J. Voigtlaender-Bolz, and S. S. Bolz. A microfluidic platform for probing small artery structure and function. *Lab Chip* 10(18):2341–2349, 2010.
[25]Hardelauf, H., J. P. Frimat, J. D. Stewart, W. Schormann, Y. Y. Chiang, P. Lampen, J. Franzke, J. G. Hengstler, C. Cadenas, L. A. Kunz-Schughart, and J. West. Microarrays for the scalable production of metabolically relevant tumour spheroids: a tool for modulating chemosensitivity traits. *Lab Chip* 11(3):419–428, 2011.
[26]Hardelauf, H., J. Sisnaiske, A. A. Taghipour-Anvari, P. Jacob, E. Drabiniok, U. Marggraf, J. P. Frimat, J. G. Hengstler, A. Neyer, C. van Thriel, and J. West. High fidelity neuronal networks formed by plasma masking with a bilayer membrane: analysis of neurodegenerative and neuroprotective processes. *Lab Chip* 11(16):2763–2771, 2011.
[27]He, M., and A. E. Herr. Automated microfluidic protein immunoblotting. *Nat. Protoc.* 5(11):1844–1856, 2010.
[28]Hirschhaeuser, F., H. Menne, C. Dittfeld, J. West, W. Mueller-Klieser, and L. A. Kunz-Schughart. Multicellular tumor spheroids: an underestimated tool is catching up again. *J. Biotechnol.* 148(1):3–15, 2010.
[29]Hsiao, A. Y., Y. S. Torisawa, Y. C. Tung, S. Sud, R. S. Taichman, K. J. Pienta, and S. Takayama. Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids. *Biomaterials* 30(16):3020–3027, 2009.
[30]Hsu, W. M., A. Carraro, K. M. Kulig, M. L. Miller, M. Kaazempur-Mofrad, E. Weinberg, F. Entabi, H. Albadawi, M. T. Watkins, J. T. Borenstein, J. P. Vacanti, and C. Neville. Liver-assist device with a microfluidics-based vascular bed in an animal model. *Ann. Surg.* 252(2):351–357, 2010.
[31]Hsu, T. H., J. L. Xiao, Y. W. Tsao, Y. L. Kao, S. H. Huang, W. Y. Liao, and C. H. Lee. Analysis of the paracrine loop between cancer cells and fibroblasts using a microfluidic chip. *Lab Chip* 11(10):1808–1814, 2011.
[32]Huang, C. P., J. Lu, H. Seon, A. P. Lee, L. A. Flanagan, H. Y. Kim, A. J. Putnam, and N. L. Jeon. Engineering microscale cellular niches for three-dimensional multicellular co-cultures. *Lab Chip* 9(12):1740–1748, 2009.
[33]Huh, D., H. Fujioka, Y. C. Tung, N. Futai, R. Paine, 3rd, J. B. Grotberg, and S. Takayama. Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. *Proc. Natl Acad. Sci. USA* 104(48):18886–18891, 2007.

[34]Huh, D., B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin, and D. E. Ingber. Reconstituting organ-level lung functions on a chip. *Science* 328(5986): 1662–1668, 2010.
[35]Hwa, A. J., R. C. Fry, A. Sivaraman, P. T. So, L. D. Samson, D. B. Stolz, and L. G. Griffith. Rat liver sinusoidal endothelial cells survive without exogenous VEGF in 3D perfused co-cultures with hepatocytes. *FASEB J.* 21(10):2564–2579, 2007.
[36]Jang, K. J., and K. Y. Suh. A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. *Lab Chip* 10(1):36–42, 2010.
[37]Kane, B. J., M. J. Zinner, M. L. Yarmush, and M. Toner. Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes. *Anal. Chem.* 78(13): 4291–4298, 2006.
[38]Khetani, S. R., and S. N. Bhatia. Microscale culture of human liver cells for drug development. *Nat. Biotechnol.* 26(1):120–126, 2008.
[39]Kim, D. H., E. A. Lipke, P. Kim, R. Cheong, S. Thompson, M. Delannoy, K. Y. Suh, L. Tung, and A. Levchenko. Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs. *Proc. Natl Acad. Sci. USA* 107(2):565–570, 2010.
[40]Kimura, H., T. Yamamoto, H. Sakai, Y. Sakai, and T. Fujii. An integrated microfluidic system for long-term perfusion culture and on-line monitoring of intestinal tissue models. *Lab Chip* 8(5):741–746, 2008.
[41]Kunze, A., M. Giugliano, A. Valero, and P. Renaud. Micropatterning neural cell cultures in 3D with a multi-layered scaffold. *Biomaterials* 32(8):2088–2098, 2011.
[42]Lecault, V., M. Vaninsberghe, S. Sekulovic, D. J. Knapp, S. Wohrer, W. Bowden, F. Viel, T. McLaughlin, A. Jarandehei, M. Miller, D. Falconnet, A. K. White, D. G. Kent, M. R. Copley, F. Taghipour, C. J. Eaves, R. K. Humphries, J. M. Piret, and C. L. Hansen. High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. *Nat. Methods* 8(7):581–586, 2011.
[43]Leclerc, E., R. Baudoin, A. Corlu, L. Griscom, J. Luc Duval, and C. Legallais. Selective control of liver and kidney cells migration during organotypic cocultures inside fibronectin-coated rectangular silicone microchannels. *Biomaterials* 28(10):1820–1829, 2007.
[44]Leclerc, E., B. David, L. Griscom, B. Lepioufle, T. Fujii, P. Layrolle, and C. Legallaisa. Study of osteoblastic cells in a microfluidic environment. *Biomaterials* 27(4):586–595, 2006.
[45]Lecuit, T., and L. Le Goff. Orchestrating size and shape during morphogenesis. *Nature* 450(7167):189–192, 2007.
[46]Lee, P. J., P. J. Hung, and L. P. Lee. An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. *Biotechnol. Bioeng.* 97(5):1340–1346, 2007.
[47]Li, A. P. In vitro evaluation of human xenobiotic toxicity: scientific concepts and the novel integrated discrete multiple cell co-culture (IdMOC) technology. *ALTEX* 25(1):43–49, 2008.
[48]Li, A. P. The use of the Integrated Discrete Multiple Organ Co-culture (IdMOC) system for the evaluation of multiple organ toxicity. *Altern. Lab Anim.* 37(4):377–385, 2009.
[49]Li, A. P., C. Bode, and Y. Sakai. A novel in vitro system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast cancer cells. *Chem. Biol. Interact.* 150(1):129–136, 2004.
[50]Liu, T., B. Lin, and J. Qin. Carcinoma-associated fibroblasts promoted tumor spheroid invasion on a microfluidic 3D co-culture device. *Lab Chip* 10(13):1671–1677, 2010.
[51]Lovchik, R. D., F. Bianco, N. Tonna, A. Ruiz, M. Matteoli, and E. Delamarche. Overflow microfluidic networks for open and closed cell cultures on chip. *Anal. Chem.* 82(9):3936–3942, 2010.
[52]Ma, H., T. Liu, J. Qin, and B. Lin. Characterization of the interaction between fibroblasts and tumor cells on a microfluidic co-culture device. *Electrophoresis* 31(10): 1599–1605, 2010.
[53]Mack, P. J., Y. Zhang, S. Chung, V. Vickerman, R. D. Kamm, and G. Garcia-Cardena. Biomechanical Regulation of Endothelium-dependent Events Critical for Adaptive Remodeling. *J. Biol. Chem.* 284(13):8412–8420, 2009.
[54]Moraes, C., J. H. Chen, Y. Sun, and C. A. Simmons. Microfabricated arrays for high-throughput screening of cellular response to cyclic substrate deformation. *Lab Chip* 10(2):227–234, 2010.
[55]Moraes, C., Y. Sun, and C. A. Simmons. (Micro)managing the mechanical microenvironment. *Integr. Biol. (Camb)* 3(10):959–971, 2011.
[56]Moraes, C., G. Wang, Y. Sun, and C. A. Simmons. A microfabricated platform for high-throughput unconfined compression of micropatterned biomaterial arrays. *Biomaterials* 31(3):577–584, 2010.
[57]Nalayanda, D. D., C. Puleo, W. B. Fulton, L. M. Sharpe, T. H. Wang, and F. Abdullah. An open-access microfluidic model for lung-specific functional studies at an air–liquid interface. *Biomed. Microdevices* 11(5):1081–1089, 2009.
[58]Nilsson, J., M. Evander, B. Hammarstrom, and T. Laurell. Review of cell and particle trapping in microfluidic systems. *Anal. Chim. Acta* 649(2):141–157, 2009.
[59]Ohashi, K., T. Yokoyama, M. Yamato, H. Kuge, H. Kanehiro, M. Tsutsumi, T. Amanuma, H. Iwata, J. Yang, T. Okano, and Y. Nakajima. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. *Nat. Med.* 13(7):880–885, 2007.
[60]Ottesen, E. A., J. W. Hong, S. R. Quake, and J. R. Leadbetter. Microfluidic digital PCR enables multigene analysis of individual environmental bacteria. *Science* 314(5804):1464–1467, 2006.
[61]Paguirigan, A. L., J. P. Puccinelli, X. Su, and D. J. Beebe. Expanding the available assays: adapting and validating in-cell westerns in microfluidic devices for cell-based assays. *Assay Drug Dev. Technol.* 8(5):591–601, 2010.
[62]Pan, W., W. Chen, and X. Jiang. Microfluidic Western blot. *Anal. Chem.* 82(10):3974–3976, 2010.
[63]Park, J., H. Koito, J. Li, and A. Han. Microfluidic compartmentalized co-culture platform for CNS axon myelination research. *Biomed. Microdevices* 11(6):1145–1153, 2009.
[64]Park, J., Y. Li, F. Berthiaume, M. Toner, M. L. Yarmush, and A. W. Tilles. Radial flow hepatocyte bioreactor using stacked microfabricated grooved substrates. *Biotechnol. Bioeng.* 99(2):455–467, 2008.
[65]Park, J. Y., S. Takayama, and S. H. Lee. Regulating microenvironmental stimuli for stem cells and cancer cells using microsystems. *Integr. Biol. (Camb)* 2(5–6):229–240, 2010.

[66]Pelosi, P. The forgotten sides of acute lung injury and acute respiratory distress syndrome. *Curr. Opin. Crit. Care* 14(1):1–2, 2008.
[67]Price, G. M., K. H. Wong, J. G. Truslow, A. D. Leung, C. Acharya, and J. Tien. Effect of mechanical factors on the function of engineered human blood microvessels in microfluidic collagen gels. *Biomaterials* 31(24):6182–6189, 2010.
[68]Rhee, S. W., A. M. Taylor, C. H. Tu, D. H. Cribbs, C. W. Cotman, and N. L. Jeon. Patterned cell culture inside microfluidic devices. *Lab Chip* 5(1):102–107, 2005.
[69]Sekiya, S., M. Muraoka, T. Sasagawa, T. Shimizu, M. Yamato, and T. Okano. Three-dimensional cell-dense constructs containing endothelial cell-networks are an effective tool for in vivo and in vitro vascular biology research. *Microvasc. Res.* 80(3):549–551, 2010.
[70]Shao, J., L. Wu, J. Wu, Y. Zheng, H. Zhao, Q. Jin, and J. Zhao. Integrated microfluidic chip for endothelial cells culture and analysis exposed to a pulsatile and oscillatory shear stress. *Lab Chip* 9(21):3118–3125, 2009.
[71]Shein, M., A. Greenbaum, T. Gabay, R. Sorkin, M. David-Pur, E. Ben-Jacob, and Y. Hanein. Engineered neuronal circuits shaped and interfaced with carbon nanotube microelectrode arrays. *Biomed. Microdevices* 11(2):495–501, 2009.
[72]Shimizu, H., K. Ohashi, R. Utoh, K. Ise, M. Gotoh, M. Yamato, and T. Okano. Bioengineering of a functional sheet of islet cells for the treatment of diabetes mellitus. *Biomaterials* 30(30):5943–5949, 2009.
[73]Shin, Y., J. S. Jeon, S. Han, G. S. Jung, S. Shin, S. H. Lee, R. Sudo, R. D. Kamm, and S. Chung. In vitro 3D collective sprouting angiogenesis under orchestrated ANG-1 and VEGF gradients. *Lab Chip* 11(13):2175–2181, 2011.
[74]Sim, W. Y., S. W. Park, S. H. Park, B. H. Min, S. R. Park, and S. S. Yang. A pneumatic micro cell chip for the differentiation of human mesenchymal stem cells under mechanical stimulation. *Lab Chip* 7(12):1775–1782, 2007.
[75]Sin, A., K. C. Chin, M. F. Jamil, Y. Kostov, G. Rao, and M. L. Shuler. The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. *Biotechnol. Prog.* 20(1):338–345, 2004.
[76]Song, J. W., S. P. Cavnar, A. C. Walker, K. E. Luker, M. Gupta, Y. C. Tung, G. D. Luker, and S. Takayama. Microfluidic endothelium for studying the intravascular adhesion of metastatic breast cancer cells. *PLoS One* 4(6):e5756, 2009.
[77]Song, J. W., W. Gu, N. Futai, K. A. Warner, J. E. Nor, and S. Takayama. Computer-controlled microcirculatory support system for endothelial cell culture and shearing. *Anal. Chem.* 77(13):3993–3999, 2005.
[78]Srigunapalan, S., C. Lam, A. R. Wheeler, and C. A. Simmons. A microfluidic membrane device to mimic critical components of the vascular microenvironment. *Biomicrofluidics* 5(1):13409, 2011.
[79]Stroock, A. D., and C. Fischbach. Microfluidic culture models of tumor angiogenesis. *Tissue Eng. A* 16(7):2143–2146, 2010.
[80]Sung, J. H., C. Kam, and M. L. Shuler. A microfluidic device for a pharmacokinetic-pharmacodynamic (PK-PD) model on a chip. *Lab Chip* 10(4):446–455, 2010.
[81]Sung, J. H., and M. L. Shuler. A micro cell culture analog (microCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anticancer drugs. *Lab Chip* 9(10):1385–1394, 2009.
[82]Tavana, H., A. Jovic, B. Mosadegh, Q. Y. Lee, X. Liu, K. E. Luker, G. D. Luker, S. J. Weiss, and S. Takayama. Nanolitre liquid patterning in aqueous environments for spatially defined reagent delivery to mammalian cells. *Nat. Mater.* 8(9):736–741, 2009.
[83]Tavana, H., C. H. Kuo, Q. Y. Lee, B. Mosadegh, D. Huh, P. J. Christensen, J. B. Grotberg, and S. Takayama. Dynamics of liquid plugs of buffer and surfactant solutions in a micro-engineered pulmonary airway model. *Langmuir* 26(5):3744–3752, 2010.
[84]Tavana, H., P. Zamankhan, P. J. Christensen, J. B. Grotberg, and S. Takayama. Epithelium damage and protection during reopening of occluded airways in a physiologic microfluidic pulmonary airway model. *Biomed. Microdevices* 13(4):731–742, 2011.
[85]Tilles, A. W., F. Berthiaume, M. L. Yarmush, R. G. Tompkins, and M. Toner. Bioengineering of liver assist devices. *J. Hepatobiliary Pancreat. Surg.* 9(6):686–696, 2002.
[86]Toda, M., K. Yamamoto, N. Shimizu, S. Obi, S. Kumagaya, T. Igarashi, A. Kamiya, and J. Ando. Differential gene responses in endothelial cells exposed to a combination of shear stress and cyclic stretch. *J. Biotechnol.* 133(2):239–244, 2008.
[87]Toh, Y. C., T. C. Lim, D. Tai, G. Xiao, D. van Noort, and H. Yu. A microfluidic 3D hepatocyte chip for drug toxicity testing. *Lab Chip* 9(14):2026–2035, 2009.
[88]Toh, Y. C., C. Zhang, J. Zhang, Y. M. Khong, S. Chang, V. D. Samper, D. van Noort, D. W. Hutmacher, and H. Yu. A novel 3D mammalian cell perfusion-culture system in microfluidic channels. *Lab Chip* 7(3):302–309, 2007.
[89]Torisawa, Y. S., B. Mosadegh, T. Bersano-Begey, J. M. Steele, K. E. Luker, G. D. Luker, and S. Takayama. Microfluidic platform for chemotaxis in gradients formed by CXCL12 source-sink cells. *Integr. Biol. (Camb)* 2(11–12):680–686, 2010.
[90]Tumarkin, E., L. Tzadu, E. Csaszar, M. Seo, H. Zhang, A. Lee, R. Peerani, K. Purpura, P. W. Zandstra, and E. Kumacheva. High-throughput combinatorial cell co-culture using microfluidics. *Integr. Biol.* 3(6):653–662, 2011.
[91]Unger, M. A., H. P. Chou, T. Thorsen, A. Scherer, and S. R. Quake. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science* 288(5463):113–116, 2000.
[92]van der Meer, A. D., K. Vermeul, A. A. Poot, J. Feijen, and I. Vermes. A microfluidic wound-healing assay for quantifying endothelial cell migration. *Am. J. Physiol. Heart Circ. Physiol.* 298(2):H719–H725, 2010.
[93]van Midwoud, P. M., M. T. Merema, E. Verpoorte, and G. M. Groothuis. A microfluidic approach for in vitro assessment of interorgan interactions in drug metabolism using intestinal and liver slices. *Lab Chip* 10(20):2778–2786, 2010.
[94]Verbridge, S. S., N. W. Choi, Y. Zheng, D. J. Brooks, A. D. Stroock, and C. Fischbach. Oxygen-controlled three-dimensional cultures to analyze tumor angiogenesis. *Tissue Eng. A* 16(7):2133–2141, 2010.
[95]Vickerman, V., J. Blundo, S. Chung, and R. Kamm. Design, fabrication and implementation of a novel multi-parameter control microfluidic platform for three-dimensional cell culture and real-time imaging. *Lab Chip* 8(9):1468–1477, 2008.
[96]Viravaidya, K., and 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):590–597, 2004.

[97]Viravaidya, K., A. Sin, and M. L. Shuler. Development of a microscale cell culture analog to probe naphthalene toxicity. *Biotechnol. Prog*. 20(1):316–323, 2004.
[98]Vishwanathan, A., G. Q. Bi, and H. C. Zeringue. Ring-shaped neuronal networks: a platform to study persistent activity. *Lab Chip* 11(6):1081–1088, 2011.
[99]Walsh, C. L., B. M. Babin, R. W. Kasinskas, J. A. Foster, M. J. McGarry, and N. S. Forbes. A multipurpose microfluidic device designed to mimic microenvironment gradients and develop targeted cancer therapeutics. *Lab Chip* 9(4):545–554, 2009.
[100]Wang, Y., R. Dhopeshwarkar, R. Najdi, M. L. Waterman, C. E. Sims, and N. Allbritton. Microdevice to capture colon crypts for in vitro studies. *Lab Chip* 10(12):1596–1603, 2010.
[101]Wang, Y., J. F. Lo, J. E. Mendoza-Elias, A. F. Adewola, T. A. Harvat, K. P. Kinzer, D. Lee, M. Qi, D. T. Eddington, and J. Oberholzer. Application of microfluidic technology to pancreatic islet research: first decade of endeavor. *Bioanalysis* 2(10):1729–1744, 2010.
[102]Wei, C. W., J. Y. Cheng, and T. H. Young. Elucidating in vitro cell-cell interaction using a microfluidic coculture system. *Biomed. Microdevices* 8(1):65–71, 2006.
[103]Williams, C. H., and C. C. Hong. Multi-step usage of in vivo models during rational drug design and discovery. *Int. J. Mol. Sci*. 12(4):2262–2274, 2011.
[104]Wlodkowic, D., and Z. Darzynkiewicz. Rise of the micromachines: microfluidics and the future of cytometry. *Methods Cell Biol*. 102:105–125, 2011.
[105]Wu, L. Y., D. Di Carlo, and L. P. Lee. Microfluidic self-assembly of tumor spheroids for anticancer drug discovery. *Biomed. Microdevices* 10(2):197–202, 2008.
[106]Yang, J., M. Yamato, T. Shimizu, H. Sekine, K. Ohashi, M. Kanzaki, T. Ohki, K. Nishida, and T. Okano. Reconstruction of functional tissues with cell sheet engineering. *Biomaterials* 28(34):5033–5043, 2007.
[107]Zhang, C., Z. Zhao, N. A. Abdul Rahim, D. van Noort, and H. Yu. Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. *Lab Chip* 9(22):3185–3192, 2009.
[108]Zheng, Y., P. W. Henderson, N. W. Choi, L. J. Bonassar, J. A. Spector, and A. D. Stroock. Microstructured templates for directed growth and vascularization of soft tissue in vivo. *Biomaterials* 32(23):5391–5401, 2011.