ANNUAL REVIEWS

Annual Review of Analytical Chemistry

Clinically Relevant Tissue Scale Responses as New Readouts from Organs-on-a-Chip for Precision Medicine

Olivier T. Guenat,[1,2,3] Thomas Geiser,[2] and François Berthiaume[4]

[1]ARTORG Center for Biomedical Engineering Research, Medical Faculty, University of Bern, CH-3008 Bern, Switzerland; email: olivier.guenat@artorg.unibe.ch

[2]Department of Pulmonary Medicine, University Hospital and University of Bern, CH-3008 Bern, Switzerland

[3]Thoracic Surgery Department, University Hospital of Bern, Switzerland

[4]Department of Biomedical Engineering, Rutgers University, Piscataway, New Jersey 08854, USA

Annu. Rev. Anal. Chem. 2020. 13:111–33

First published as a Review in Advance on January 21, 2020

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

https://doi.org/10.1146/annurev-anchem-061318-114919

Keywords

organ-on-a-chip, tissue function, tissue response, clinical application, microenvironment, precision medicine

Abstract

Organs-on-chips (OOC) are widely seen as being the next generation in vitro models able to accurately recreate the biochemical-physical cues of the cellular microenvironment found in vivo. In addition, they make it possible to examine tissue-scale functional properties of multicellular systems dynamically and in a highly controlled manner. Here we summarize some of the most remarkable examples of OOC technology's ability to extract clinically relevant tissue-level information. The review is organized around the types of OOC outputs that can be measured from the cultured tissues and transferred to clinically meaningful information. First, the creation of functional tissues-on-chip is discussed, followed by the presentation of tissue-level readouts specific to OOC, such as morphological changes, vessel formation and function, tissue properties, and metabolic functions. In each case, the clinical relevance of the extracted information is highlighted.

1. INTRODUCTION

Organ-on-a-chip (OOC) technology is emerging as a credible alternative to animal models that avoids some of the shortcomings of standard cell-based assays and whole organisms (1, 2). OOCs are microengineered cell-based systems that provide cells with an environment that closely resembles their native in vivo milieu. These systems are based on microfabricated structures with size scales similar to those of individual cells or cell aggregates and can replicate the topographical, chemical, and physical cues of the cellular microenvironment. Moreover, OOC technology affords the possibility to examine dynamic aspects of tissue physiology in a highly controlled manner, by modulating system inputs as a function of time, studying tissue responses dynamically, or both. Time-varying inputs such as flow, biochemical gradient, compression force, or cyclic stretching can be precisely tuned to engineer the organ-specific environment. This in vivo–like milieu enables cells to maintain their native phenotype or differentiate into a specific phenotype. This, in turn, allows the creation of functional tissues that emulate complex biological functions. On the output side, the ability to monitor the evolution of the biological response over time can potentially provide much more information than a snapshot measurement. This is the equivalent of longitudinal in vivo studies, but unlike in vivo systems that have limited parameters that can be studied without sacrificing the animal, the OOC model can be engineered to provide detailed mechanistic information. Furthermore, although OOC models cannot (yet) reproduce the whole in vivo intricacy, one of their major advantages versus in vivo models is the possibility to identify, record, and assess the effects of a singular parameter (force, flow, specific cell type, etc.) on the biological model by adding or subtracting it to/from the system in a functional way. Similarly, by excluding confounding multiorgan interactions, one can better characterize the function of a specific tissue. Multi-OOC systems are also being developed where those interactions can be studied in a controlled manner (3).

Standard cell culture models are designed to provide uniform conditions in order to facilitate data interpretation, as all cells in a culture well are assumed to respond identically to the stimulus. Furthermore, the culture medium volume-to-cell ratio is typically large to avoid the impracticality of frequent medium changes. This scenario is very different from in vivo conditions, where cells in different locations of a tissue are exposed to different levels of oxygen, hormones, physical stresses, etc. Thus, tissue formation and function are poorly reproduced under such conditions. Furthermore, in vivo tissues are afforded a continuous nutrient supply from blood, which makes it possible to function at much higher cell numbers per unit volume than in vitro. Consequently, metabolites accumulate in much higher levels than they do in vitro, and metabolic gradients that typically form within tissues are not seen in standard culture models.

Thus, in sharp contrast to standard cell culture models, OOC technology does not just provide subcellular and cellular information but it affords the possibility to extract tissue-level information as a function of time. OOCs have been shown to recapitulate intricate multifactorial effects, such as the immune response to microbial infection in human lung alveoli (4), the vasoactive response of a 3D microvasculature lined with patients' pericytes exposed to a vasoconstrictor (5), thrombosis formation (6), or tumor cell death in a spheroid induced by an antineoplastic drug metabolized by a 3D hepatocyte culture (7). In the above examples, the comprehensive recapitulation of lung infection and inflammation is the consequence of a cascade of biological events where the flow played a major role (more details are provided in Section 3.1 on vessel functions). The recapitulation of such a complex organ-level process is a breakthrough and paradigm shift in cell biology and represents a major milestone in the cell culture history. It is anticipated that in the near future the information provided by OOCs may approach that of human clinical data. We anticipate that data gained from in vitro systems will thus no longer give information solely about cellular

functions but also about tissue-level and even whole organism-level functions in healthy and diseased human tissues.

This review focuses on the amazing possibility provided by OOC technology to extract clinically relevant tissue-level information from in vitro systems. It is structured around outputs that can be measured from the cultured tissues in OOCs that deliver information about morphology (e.g., tissue growth, vessel formation) and tissue functions and properties (e.g., metabolic, endocrine, mechanical, and electrical). Special attention is given to vascularization of OOCs, a critical feature that overcomes diffusion-limited transport in standard cultures and enables the culture of tissue-like multicellular structures. We also discuss future challenges and opportunities in the field.

2. COMPLEX AND FUNCTIONAL TISSUE FORMATION ON CHIP

Complex, more physiological, and functional models have emerged owing to three major innovations: (*a*) the use of biologically relevant cells, (*b*) the availability of various and specific physiological media, and (*c*) the possibility to create in vivo–like cellular microenvironments, in which cells feel like they would in their native milieu. First, cell lines (8) that have been broadly used owing to their hardiness and immortal characteristics are being replaced by cells whose phenotype better resembles that found in vivo. Primary cells, which are so far the best available cell models, and induced pluripotent stem cells (iPSCs) are receiving much attention. In a precision medicine context, the limited life span and the scarcity of primary cells are some of the main challenges of these cells. In contrast, iPSCs, which do not suffer these drawbacks, are widely seen as the ideal cell source to create physiological and disease-specific relevant models (9) and enable parallel screening of potentially therapeutic compounds. In addition, iPSC technology will allow the creation of a variety of patient-specific cells (endothelial cells, epithelial cells, fibroblasts, etc.) that can be used in multicellular models representing the in vivo situation of an individual patient. However, although enormous progress has been made in their development, further development is slowed until a broad range of organ-specific iPSCs that fully mimic their native phenotype and morphology becomes available. Second, the growth, differentiation, and culture of these cells are being made possible via a better understanding of the various biochemical factors, including the extracellular matrix, involved in these processes (10) and the availability of specific cell culture media (11). Finally, the advent of microfluidic 3D environments engineered using microfabrication techniques (12) makes it possible to produce in vivo–like cellular microenvironments, of which the geometry, material properties, and flow conditions can accurately be controlled.

The functions of a tissue vary with scale and time (13). At the cellular level, the function of a tissue is to provide an environment that enables cells to maintain their specific phenotypes and functions. Supplied with nutrients and oxygen via a dense network of capillaries and triggered by time-dependent environmental biophysical-chemical cues, cells self-organize into structures of higher order. Tissues with specific properties are created—a muscle, a microvessel, a liver sinusoid—and are capable of emulating a tissue-scale response, muscle contraction, vessel sprouting, and bile production.

As described above, the creation of functional tissues on a chip requires functional cells (**Figure 1**), suitable cell culture media, and an in vivo–like environment specific to the tissue of interest. Recent progress in microfabrication and microfluidic technologies has made it possible to produce complex tissues that can be maintained in culture for several weeks. A new generation of OOCs is being developed at the frontier of tissue engineering, microfluidics, and material sciences. The following examples illustrate recently reported microstructured scaffolds used to create complex and functional tissues on a chip. A microstructured collagen scaffold exposed to

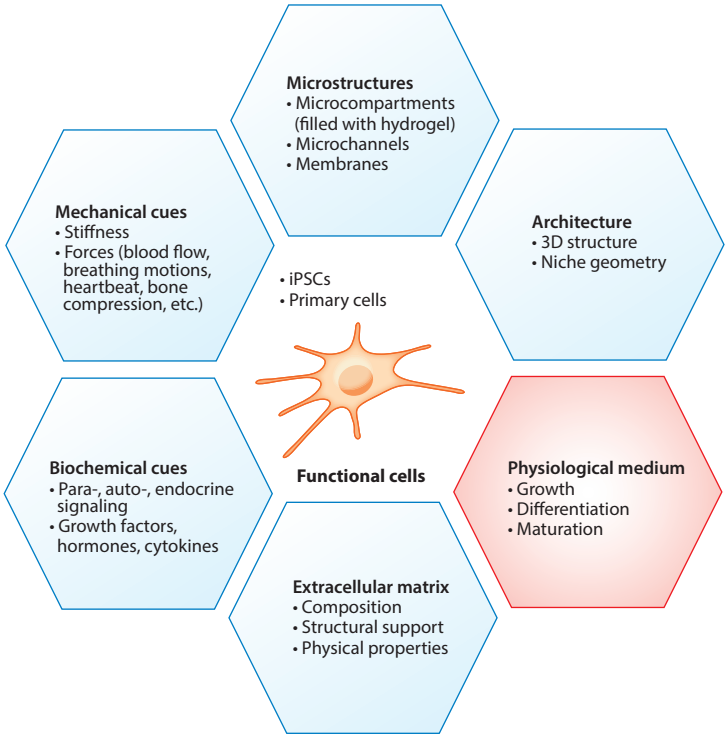

Figure 1

The microenvironment of functional cells. The dynamic process of the formation of complex and functional tissues on a chip requires functional cells, the proper cellular microenvironment (*blue*) specific to the organ to be emulated, and specialized cell culture media (*red*). Abbreviation: iPSC, induced pluripotent stem cell.

a growth factor gradient was used to generate a high density of human colon crypts mimicking the architecture, luminal accessibility, tissue polarity, cell migration, and cellular responses of in vivo intestinal crypts (14). The generation of bone marrow was reported using a hydroxyapatite-coated zirconium oxide ceramic scaffold, enabling successful culture of hematopoietic stem and progenitor cells for four weeks (15). An array of lung alveoli with in vivo–like dimensions using a collagen-elastin stretchable membrane was reported, on which lung alveolar epithelial cells from patients were cultured for several weeks (16). A biodegradable scaffold was used to engineer a network of perfusable microvessels, which, in turn, was used to seed organ-specific parenchymal cells, enabling extensive remodeling that led to the creation of vascularized hepatic and cardiac tissues (17). The complexity of tissues that can be created on chip is continuing to increase. Recently, the successful vascularization of spheroids (18) and of organoids-on-chip (19, 20) illustrates the potential of combining organoid and OOC technologies (21, 22).

3. NEW READOUTS OF TISSUE FUNCTIONS IN HEALTHY AND DISEASED STATES

The discovery of the stethoscope 200 years ago by Laennec is an emblematic milestone in the development of medical devices (23). More than any other medical instrument, the stethoscope symbolizes medical examination and diagnosis. It enables hearing internal sounds from the heart, lungs, intestine, and larger blood vessels and helps identify pathophysiological changes, such as

abnormal heart sounds or wheezing or crackling in the lungs, which was among the first tissue-level functions to be reproduced on chip (24). Today, various medical instruments can extract a multitude of information at resolutions that may soon reach 100 μm (25) in tissues located deep inside the human body. As OOCs start to emulate complex and functional tissues, the prospect of obtaining information from a tissue cultured in vitro that can be correlated to clinical information becomes a reality. Moreover, when the cultured tissue originates from the patient's own cells, combining the in vitro data with the clinical data opens completely new avenues in precision medicine. Information obtained by OOC technology using patient-specific cells may be helpful for choosing drugs that may be most efficient while having the fewest side effects in the respective patient. OOC technology may therefore be relevant not only in diagnosis but also in pharmacological decision making and prognosis in the individual patient. Changes in tissue functions can be monitored to trace tissue growth, pathogenesis, or toxic insults. **Figure 2** illustrates a symbolic

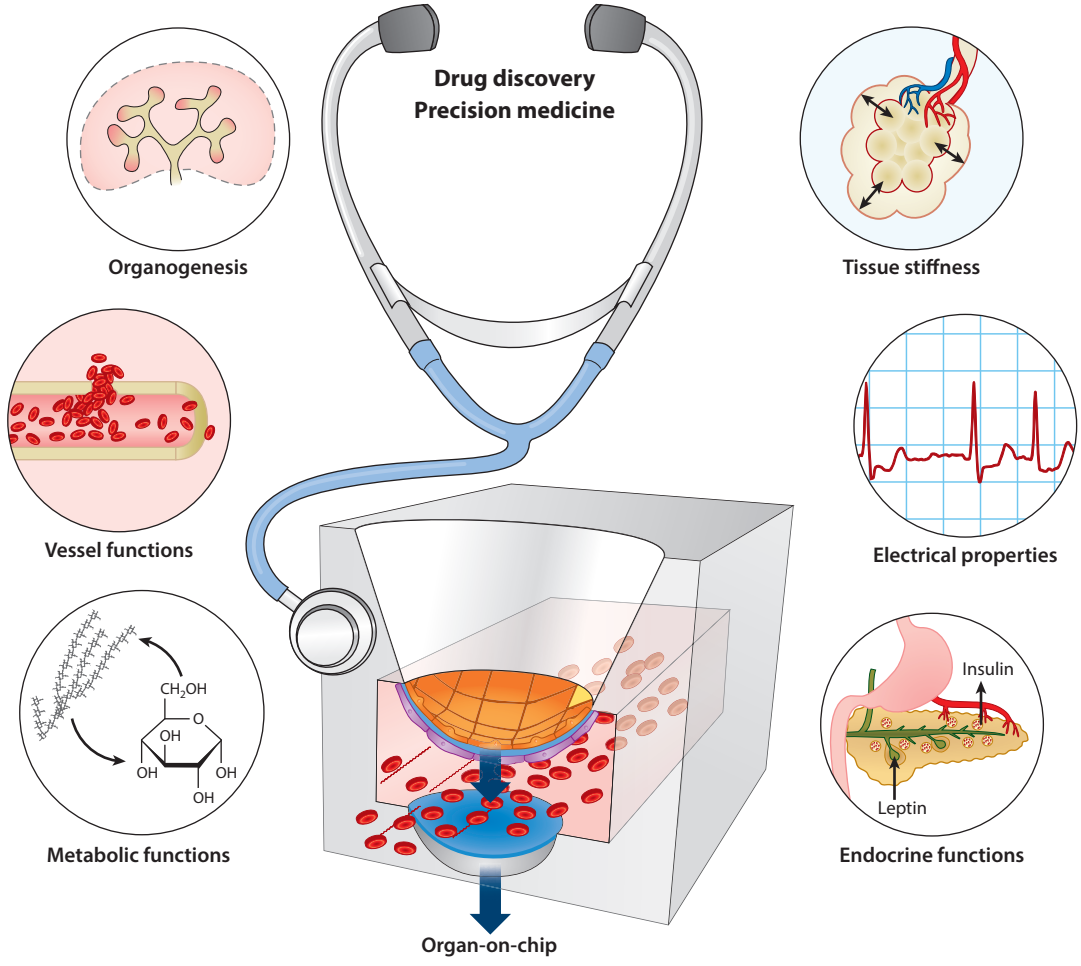

Figure 2

Tissue-scale responses in organs-on-chip. A symbolic stethoscope monitors an organ-on-chip (lung-on-chip), detecting changes in tissue functions and properties that are clinically relevant and may render information that can be used in a precision medicine approach to individual patient care.

stethoscope "auscultating" an OOC to monitor tissue properties or responses, such as growth, metabolic imbalance, cardiac tissue arrhythmias, secretion (paracrine or autocrine), and/or tissue stiffening. Advanced cell and tissue characterization regarding structure, mechanics, and composition will result in improved tissue-informed engineering and precision biomaterial development, allowing personalized in vitro disease models for precision medicine (26). After validation, these organotypic in vitro models using patient-specific cells may have diagnostic or even prognostic value and may help drug screening, improve patient care and clinical outcomes, and contribute to cost reduction in health care from using a precision medicine approach.

3.1. Vessel Functions

The transport of fluids in the body is a vital multiscale phenomenon that takes place at both the micro- and macroscopic levels. It is central to most if not all biological events. The osmotic regulation of cell volume, recruitment of immune cells to the inflammation site, the angiogenic process, and breathing movements are just a few examples of many occurrences where fluid transport is involved. While predominantly diffusive in small pores, the fluid flow is mostly convective in bigger vessels. Large and small vessels, often organized in networks, convey different types of fluids: blood, air, water, cells, secretions, or waste products. Although the circulatory and lymphatic systems spread throughout the entire body, other vessel networks are dedicated to a specific organ or interact with a limited number of organs. Their anatomy and cellular physiology vary greatly and are organ dependent. In the lung, for example, the respiratory tree is a vast network of air and blood vessels, responsible for the transport of oxygen and the removal of carbon dioxide, among other functions. The digestive system, with its tubes of various cross sections and morphologies, is in charge of the transport, digestion, and absorption of nutrients, and of the formation and extraction of feces. The endocrine system is composed of a number of glands secreting specific fluids, such as saliva, sweat, insulin, and adrenaline, to name a few, whose effects can be local and/or systemic.

3.1.1. Blood vessel formation. One of the most striking successes of OOC technology is undoubtedly the development of perfusable endothelialized vessels. 3D angiogenic assays created in standard dishes are able to reproduce early angiogenic stages, such as the apico-basal endothelial polarity and the formation of hollow cyst-like channels (27). However, they are unable to recapitulate the functions of mature vessels due to the impossibility of perfusing lumens. In sharp contrast, microfluidics makes it possible to create long-lived and functional endothelial vessels that can be investigated in a dynamic setting for longer periods of time. The key aspect of this masterpiece is the ability to reproduce in vivo–like cues—biochemical gradients or mechanical stimuli (interstitial flow, shear stress)—necessary to create open and perfusable vessels.

These systems are created either by patterning channels wrapped with endothelial cells or by endothelial cell self-assembly resulting in microvessel networks. Haase & Kamm (28) recently reviewed the various microfluidic designs aimed at studying angiogenesis and vasculogenesis and the role of the cellular microenvironment in the creation of functional vessels. Since then, the interest in microvascularized models has continued to grow. The identity and specific functions of the pericytes and the importance of their integration in microvascular networks have also been discussed (29). These systems are widely seen as building blocks to vascularize tissues. The vascularization of tumor spheroids sparked a lot of attention because they make it possible to develop more predictive cancer models that take the tumor vascular network into account (30). A new simple and versatile microfluidic system aimed at vascularizing tumor spheroids was reported by Jeon and

colleagues (31). The system enables investigating the vascularized tumor microenvironment and anticancer drug screening. The same system was used to investigate ocular neovascularization (32).

3.1.1.1. Permeability. Permeability is one of the main functions of endothelium lining the blood vessels. The semipermeable barrier finely controls the passive transport of fluids, solutes, and macromolecules between the vessels and the surrounding tissues by interstitial and/or osmotic pressure gradients. It aims at providing tissues with nutrients and oxygen to preserve their homeostasis. When this equilibrium is disturbed due to a pathological condition, such as inflammation or infection, gaps form in the endothelial layer, leading to an increased barrier permeability, an accumulation of fluids in the tissue space and, ultimately, to the formation of an edema. In their landmark article, Huh et al. (33) succeeded in reproducing drug toxicity–induced pulmonary edema. Endothelial cells were exposed to interleukin-2 (IL-2), a drug used to treat cancer patients and known to induce vascular leakages via VE-cadherin redistribution (34). In the latter case, fluid accumulation took place at the air-liquid interface, with alveolar epithelial cells cultured at the apical side (exposed to air) and an endothelial monolayer at the basolateral side (exposed to medium) of a thin, porous, and flexible membrane. A remarkable result of this study is the surprising similarity between the barrier permeability of the lung-on-chip and that of a whole mouse lung ex vivo, demonstrating the predictive potential of the OOC model. Using the same technology, Jain et al. (35) were able to recapitulate intravascular thrombosis induced by lipopolysaccharide and to inhibit its effect using an antithrombotic compound. Remarkably, the in situ thrombus formation could be monitored and quantified in real time.

Therefore, these in vitro vessel models-on-chip will allow the study of coagulation but also fibrinolysis, which will facilitate using the model for drug screening and drug selection of fibrinolytics and anticoagulants in patients suffering from cardiovascular or cerebrovascular events (myocardial infarction, stroke). To increase the clinical relevance of the assays, healthy and diseased primary cells from patients have been cultured in those models, allowing the detection of pathophysiological changes. The antivasculogenic effect of nintedanib, a triple tyrosine kinase inhibitor used to treat idiopathic pulmonary fibrosis, was tested in a microvasculature model made of primary endothelial cells and patients' fibroblasts. Nintedanib significantly increased microvascular permeability, inhibited vasculogenesis, and remodeled preexisting microvessels by shrinking and rupturing intravascular connections (36). Patient tumor-derived pericytes led to the formation of perfusable vessels with significantly increased permeability compared to their matched normal cells. They also secreted higher levels of the proinflammatory cytokine IL-6 and expressed higher levels of the immune-suppressing protein PD-L1, proof that perivascular cells can affect the function of surrounding blood vessels (37). The increased vessel permeability found in vessels made with tumor endothelial cells was correlated with a significantly larger number of angiogenic sprouts in comparison to their normal counterpart. The vessels were further used for patient-specific screening of antiangiogenic drugs (38).

Permeability is usually determined by adding molecules of specific molecular weights at the apical side of the membrane and then quantifying the concentration of molecules that crossed the barrier by absorbance, fluorescence or luminescence spectroscopy. Other methods have been reported to assess endothelial permeability (39), one being transendothelial electrical resistance (TER). This method is widely used in combination with cell culture inserts and is being adopted by the OOC community (40). It measures integrity of the tight junctions of the endothelial barrier using electrodes placed on both sides of the barrier (41). However, the integration of electrodes or microelectrodes in tiny microchannels typical for OOCs remains a challenge, even more so in fragile self-assembled perfusable vessels. Nevertheless, the prospect of getting real-time and dye-free TER information from the endothelial barrier integrity will lead to improved

technological solutions in the near future. For now, the permeability of self-assembled vessels is mostly based on the diffusion of dyes of known diffusion coefficients across the vessel wall and the hydrogel in which they are formed. Interestingly, recent findings suggest that permeability values obtained with self-assembled microvascular networks better reflect in-vivo values than those obtained from endothelial monolayer cultured on transwell systems (42). The presence of a functional endothelium, in particular the presence of continuous tight junctions between endothelial cells and of a functional glycocalyx, was thought to be the reason of the permeability value difference.

3.1.1.2. Cell infiltration. The endothelial barrier is also permissive to active cell transport, which involves cell–cell adhesion molecules, release of cytokines, and cytoskeletal-driven cell movement through the endothelium. This active and complex process is central to a number of biological events, such as inflammation, where it involves leukocyte infiltration (43), and metastasis formation, via migration of cancer cells from the tissue into the intravascular space (44). The cascade or part of the cascade of these events could recently be reproduced in OOC systems, as summarized in the following examples. In a blood-brain barrier-on-chip, the tightness of a vascular vessel made of human brain microvascular endothelial cells was compromised following a tumor necrosis factor-alpha (TNF-α)-induced inflammatory response. As a result, an increase of neutrophil infiltration was observed that could be stopped upon inhibition of the inflammatory response (45). Another study reported neutrophils' response to *Staphylococcus aureus* in a skin-on-chip model. The migration of neutrophils, obtained from whole blood, toward the infected skin tissue samples, obtained from microbiopsies, was significantly reduced upon antibiotic treatment (46). Vessel narrowing is a characteristic of atherosclerosis, a chronic inflammatory disorder, leading to cardiovascular diseases. A novel microfluidic system including a 3D stenosis was lately developed to study vascular inflammation and leukocyte-endothelial interactions. Whole blood was perfused in the constricted channel, which resulted in significant dose-dependent effects of leukocyte adhesion in healthy and inflamed (TNF-α-treated) blood samples (47). The interaction of circulating tumor cells with the endothelium is crucial in the metastatic cascade. A recent study reported on the development of a tri-culture microfluidic platform combining a channel coated with endothelial cells interfacing with a hydrogel bone microenvironment in which organ-specific metastatic potential of three different cancer cell lines was assessed. Ovarian cancer cells presented the lowest migration rate and bladder cancer cells the highest, which recapitulate their different levels of bone tropism observed in vivo (48).

Another recent study beautifully showed the transmigration of monocytes from the intravascular to the extravascular environment (**Figure 3**) and provided the first evidence of the potential of monocytes to directly reduce cancer cell extravasation (49). This represents an important step in our understanding of the metastatic cascade and provides a new screening model for immunotherapies. It also demonstrates that OOC systems can be powerful tools for identifying the effects of specific parameters of a complex system. In the clinic, the immunotherapy response is observed based on the tumor size reduction, usually according to the RECIST (50) criteria, with some modifications specific to application in immunotherapies (51). The in vivo observation of the initial stages of metastasis remains difficult due to the limited resolution at which micrometastasis can be diagnosed (52).

3.1.1.3. Vasoactivity. Vasoactivity is another important function of the endothelial barrier that regulates blood flow to match tissue metabolism (53). The vessel diameter increases or decreases upon contraction or relaxation of the pericytes or smooth muscle cells lining the endothelial wall. Günther et al. (54) demonstrated the maintenance of the vasoconstriction and

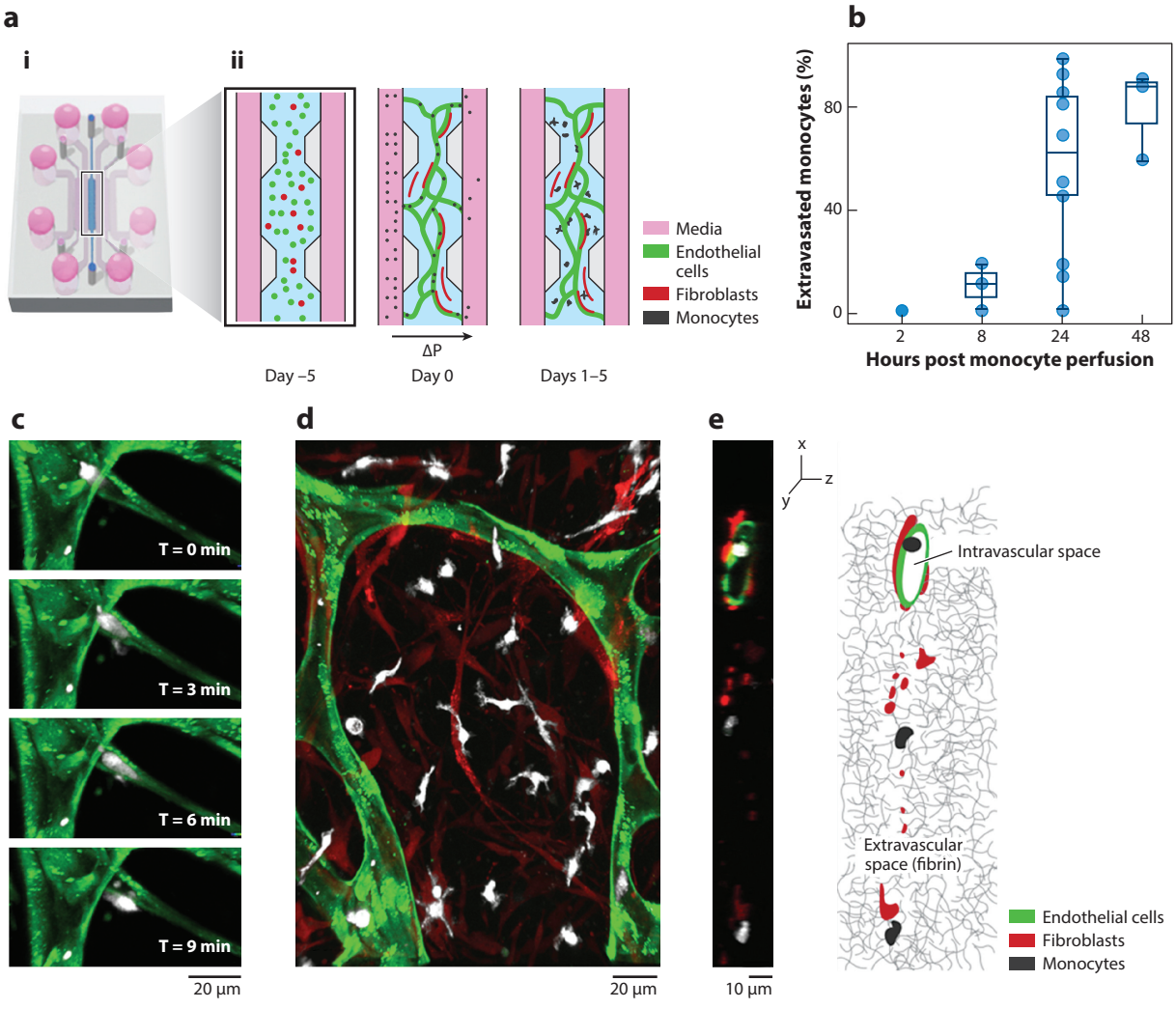

Figure 3

Microvascular cell extravasation. (*a*, *i*) Microfluidic device with a central compartment filled with gel and cells (*blue*) surrounded by two microfluidic channels filled with media (*pink*). (*a*, *ii*) Close-up of the central compartment at different time points: at day −5, endothelial cells (*green*) and fibroblasts (*red*) are suspended in a 3D fibrin gel. At day 0, endothelial cells form perfusable microvessels, in which monocytes are perfused by applying a transient pressure drop (ΔP). Within a few days (1–5), monocytes have transmigrated through the endothelial wall and are found in the extracellular fibrin gel. (*b*) Monocyte extravasation postperfusion. (*c*) Confocal images of a monocyte (*white*) undergoing extravasation through the endothelium (*green*). (*d*) Four days after perfusion. (*e*) Cross-sectional view of a vessel segment (*green*) and the extravascular space surrounding it (*right*). Figure adapted with permission from Reference 49. Copyright 2019, Elsevier.

vasodilation functions of microdissected mouse artery perfused in a microfluidic device. However, the vasoactive function of a self-assembled microvasculature-on-chip made of endothelial cells and patients' pericytes was shown only recently. Endothelial microvessels lined with patients' pericytes significantly contracted upon exposure to phenylephrine (**Figure 4**), a vasoconstrictor used to increase blood pressure in patients suffering from hypotension (55). Endothelial capillaries without pericytes did not respond (5). The vasoactive response that took place within

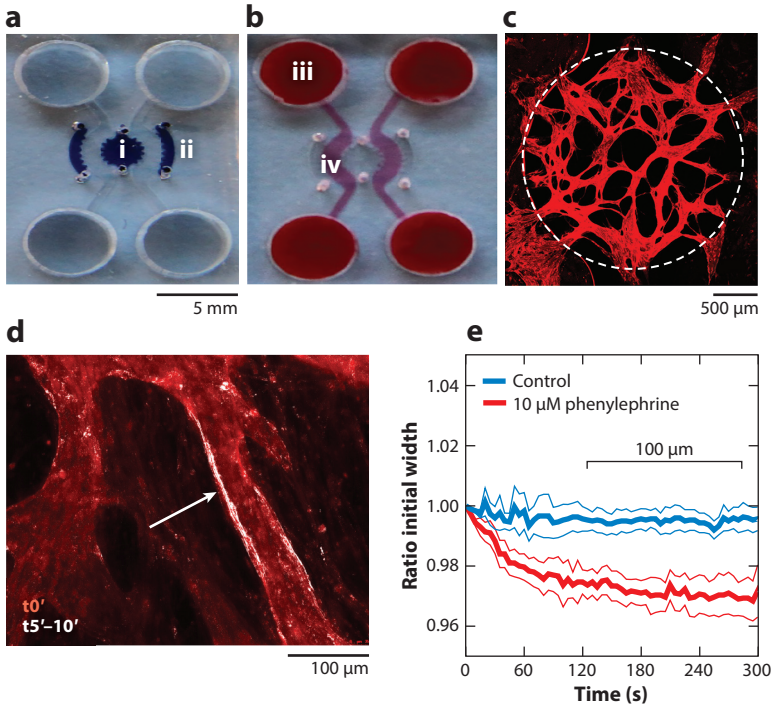

Figure 4

Vasoactive function. (*a*) Microvasculature-on-chip device with a central (*i*) and two adjacent (*ii*) compartments filled with fibrin gel and cells and (*b*) connected to each other via microchannels and their reservoirs (*iii*), filled with physiological medium (*iv*). Each reservoir is 5 mm in diameter. (*c*) In vitro microvasculature-on-chip made of endothelial cells and patient lung pericytes. (*d*) Microvasculature perfused with 10 µM phenylephrine, overlaid with the difference of images taken 5 min apart, before (*red*) and after (*white*) exposure. (*e*) Vasoconstriction effect upon administration of phenylephrine. Figure adapted with permission from Reference 5. Copyright 2015, Mary Ann Liebert, Inc.

tens of seconds was recorded by measuring the change in the vessel radius. A comparable response was recently reported by researchers using a similar microvascularized network system upon exposure to atmospheric nanoparticles (56). The vessel permeability and vasoconstriction resulting from the nanoparticle stimulation were thought to be related to vascular inflammation. Vasoactive drugs that are clinically used to modify blood pressure in the lung may be tested in a microvasculature-on-chip, depending on the type of endothelial cell used. For instance, the effect of vasoconstrictive or antihypertensive drugs or drugs used to modify pulmonary hypertension may be tested in vitro using the microvasculature-on-chip.

3.1.2. Other vessels. The formation of other vessels than blood vessels was already reproduced in OOC systems. Much research effort invested in reproducing the kidney in OOCs has been reviewed extensively (57–60). The recapitulation of a functional glomerulus (61, 62) and a functional proximal tubule (63) was achieved, not to mention the kidney excretory function in a multiorgan-on-chip system (64). The human intestine has also been investigated, particularly in regard to the interactions between the microbiome and the intestine barrier (65, 66). The development of a bile duct-on-chip that phenocopies not only the tubular architecture of the bile duct in three dimensions but also its barrier functions was recently reported (67).

3.2. Organogenesis

The remarkable self-organization property of endothelial cells and pericytes in creating self-assembled microvasculature demonstrates the potential of OOC systems to create structures of higher order. Nelson et al. (68) succeeded in a pioneering work to precisely control the mechanical environment of the fetal lung to control airway branching morphogenesis, the frequency of airway smooth muscle contraction, and the rate of developmental maturation of the lungs. The combination of OOC technology with stem cells and organoids will make it possible to better understand fundamental self-assembly mechanisms and, ultimately, organogenesis. Manfrin et al. (69) recently reported on how human pluripotent stem cell colonies exposed to spatiotemporally controlled morphogen gradients break their intrinsic radial symmetry to produce distinct axially arranged differentiation domains. Such findings are critically important, as these gradients play a key role in early in vivo embryonic development, morphogenesis, and organogenesis (70).

3.3. Tissue Stiffness

The tissue mechanical property is a key parameter of the cellular microenvironment. It is caused by the amount and the composition of the extracellular matrix. Collagen I is one of the main extracellular matrix proteins responsible for tissue stiffening and is usually quantified in vitro by colorimetric or immunoassays (71) or atomic force microscopy (AFM) (72), a cumbersome, time-consuming technique that provides a local stiffness value not necessarily indicative of the tissue stiffness. Tissue stiffness is organ specific and greatly varies in health and diseases. In the lung, for instance, the lung acellular matrix stiffness increases from 2 kPa on average (healthy lung) to about 16 kPa (fibrotic lung) (73). In lung fibrosis, the stiffening of the matrix results from an excess of fibrous connective tissue, excessive collagen deposition, and the development of fibrotic scars (74). The fibrotic process can affect many organs in the body (liver, lung, kidney, and heart) and remains difficult to treat because fibrosis is thought to be mostly irreversible, and the drug response is often patient specific. In vivo models—in particular, the current gold standard of lung fibrosis, the bleomycin model—poorly mimics the fibrotic process in humans (75).

In vitro, only parts of the fibrotic events have so far been described in OOC. The influence of breathing movements was found to significantly impair alveolar epithelial wound healing (76), a crucial phase in the fibrotic process (77). The impairment could partly be inhibited by the administration of recombinant human hepatic growth factor. The phenotypic transition from fibroblasts to myofibroblasts that are responsible for collagen synthesis (78) is another important event in the fibrotic process that has recently been shown to be influenced by mechanical compression in cardiac tissue (79).

Recently, Asmani and colleagues (80) described a simple and innovative method to detect the production of collagen I. They created arrays of micropillars, on which lung tissues—lung fibroblasts mixed with collagen—were cultured. Lung fibroblasts differentiated into myofibroblasts upon tumor growth factor (TGF)-β1 exposure, which resulted in a contraction of the suspended tissue and thus on the micropillar deflection (**Figure 5**). The system was used to predict antifibrotic response in this tissue. Treatments with pirfenidone and nintedanib, two antifibrotic drugs recently approved to treat idiopathic pulmonary fibrosis, were shown to slow down disease progression using forced vital capacity as a marker of fibrotic lung disease. They reduced tissue contractility and prevented tissue stiffening and a decline in tissue compliance (80). These results represent a significant step toward precision medicine, where patients' cells could be tested prior to starting the specific antifibrotic treatment. Further studies will be needed to compare clinical lung function measurements (forced vital capacity, lung compliance, and diffusion capacity) with information obtained by OOC or lung microtissue assays. We assume that OOCs that measure

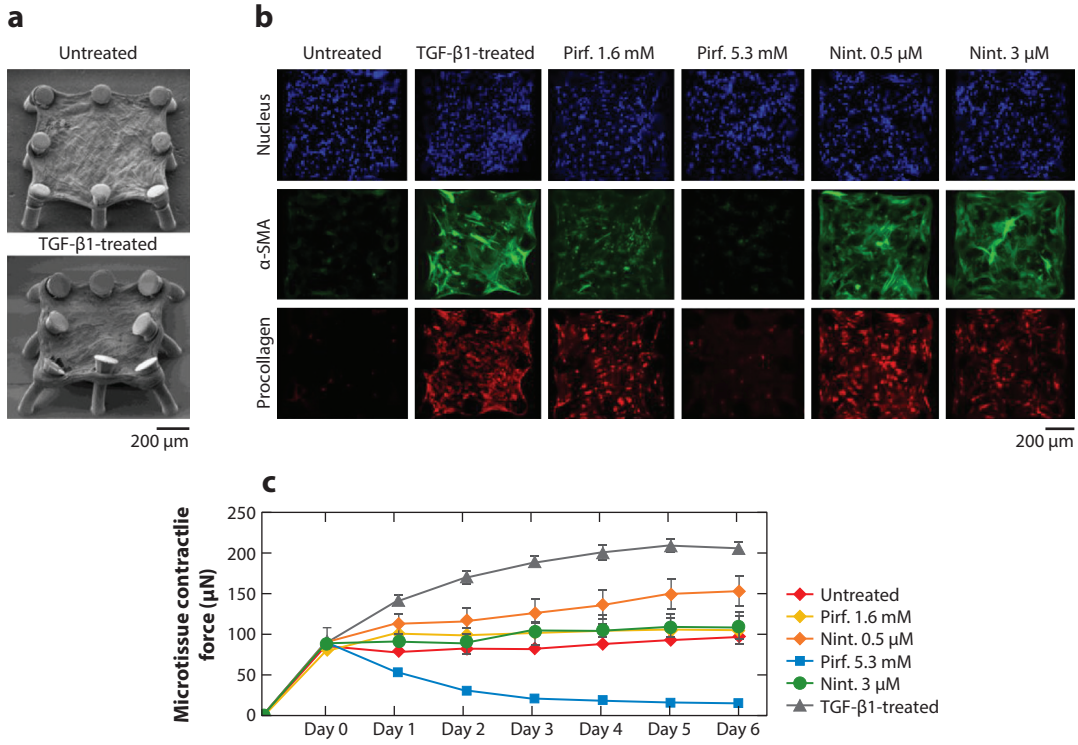

Figure 5

Tissue stiffening. Recapitulation of tissue fibrogenesis in lung microtissues. (*a*) Scanning electron microscope images of an untreated and a TGF-β1-treated microtissue. Significant micropillar deflection caused by elevated tissue contraction can be seen in TGF-β1-treated microtissue. (*b*) Antifibrotic treatment with pirfenidone (Pirf.) and nintedanib (Nint.). Immunofluorescence images of nuclei, α-SMA (alpha-smooth muscle actin), and procollagen of microtissues at day 6, with or without antifibrotic treatments. (*c*) Time-lapsed measurement of microtissue contractile force. Figure adapted with permission from Reference 91 under the terms of the Creative Commons Attribution (CC BY) License, **http://creativecommons.org/licenses/by/4.0**.

tissue stiffness may be helpful in the future for clinical decision making regarding the selection of antifibrotic treatment. Other fibrotic tissues, such as hepatic tissue, may be correlated to clinical data routinely obtained by elastography (81).

3.4. Establishing Tissue Functional Gradients: Externally Imposed Spatial Gradients and In Situ Oxygen Detection

A fundamental aspect of development in multicellular systems is the formation of spatial concentration gradients of metabolites, hormones, growth factors, and even extracellular matrix molecules, which are thought to guide the proliferation and differentiation of the tissue cells to form complex structures (82, 83). Some of these aspects have been reproduced in microfluidic systems to yield better differentiated and more in vivo–like tissues, such as in the case of the gut epithelium (84, 85). Metabolic gradients have also been shown to play a role in cancer tumor growth (86). Functional gradients also exist in terminally differentiated tissues and may be a critical feature of the organ. For example, an anaerobic environment is required to maintain the gut microbiome in the intestinal lumen, whereas the epithelial lining of the colon requires sufficient oxygen levels to survive and function; an interesting demonstration of a gut-on-chip device

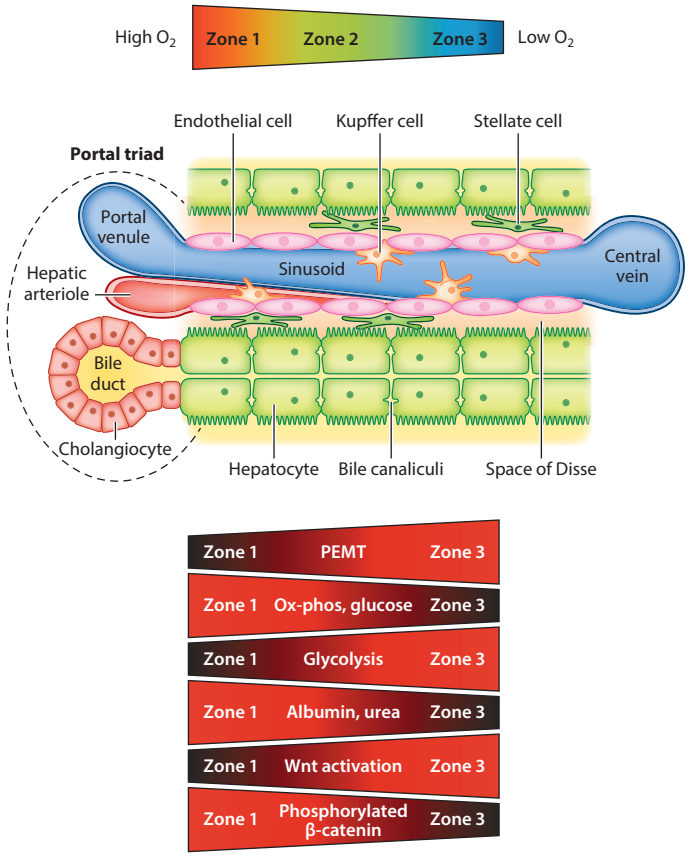

Figure 6

Liver metabolic functions. Functions vary along the length of the liver acinus. In vivo, a combination of factors, including oxygen tension and concentrations of various metabolites that vary from the portal inlet to the central vein outlet, locally modulate Wnt activation and β-catenin activity, which in turn regulate the establishment of hepatic metabolic zonation. Abbreviations: Ox-phos, oxidative phosphorylation; PEMT, phosphatidylethanolamine *N*-methyltransferase. Figure adapted with permission from Reference 88. Copyright 2017, SAGE Publications.

that recreates the anoxic-oxic interface of the colon was recently described (87). One of the best-understood tissues from the perspective of function zonation is the liver owing to its importance in drug metabolism, which we discuss in greater detail below.

The development of liver-on-chip systems is motivated by the great need for better platforms to conduct ADME/TOX (absorption, distribution, metabolism, excretion, and toxicology) studies, a crucial part of the drug discovery and development processes in the pharmaceutical industry. Although many liver microdevices have been described in the literature, a recent focus of interest is on the incorporation of functional zonation, a fundamental aspect of liver metabolism whereby different hepatocyte phenotypes, and thus metabolic processes, occur in different locations along the length of the liver acinus (**Figure 6**). Metabolic zonation impacts on major pathways of central carbon and nitrogen metabolism and on the ways drugs and environmental toxins undergo biochemical transformations in liver. There is also evidence that the initiation and early progression of certain liver disease processes, such as fatty liver and fibrosis, preferentially occur in specific zones (88).

In vivo, spatial concentration gradients form due to local consumption and production of various metabolites by the liver cells as blood flows from the periportal inlet to the perivenous outlet. It is difficult to implement a similar mechanism in microfluidic culture devices because of variability in cellular metabolism among donors, as well as viable cell density that may be attained when setting up multiple devices. An alternative approach is to externally impose the spatial gradient using a strategy called metabolic patterning on a chip, whereby different regions of the cell culture are exposed to different concentrations of metabolites from a concentration gradient generator (**Figure 7**). Using opposing gradients of hormones, such as insulin and glucagon, one can create functional gradients in glycogen storage and the urea-producing carbamoylphosphate synthetase-1 (CPS1). With a gradient of the xenobiotic 3-methylcholanthrene, a gradient of cytochrome P450 (CYP) 1A2 activity was formed, which resulted in a gradient in cell toxicity following exposure to acetaminophen (**Figure 7**). A limitation of these systems, up to this point, is that each cell region operates independent of each other, while in actual liver, metabolites that are generated by one process upstream in the liver acinus will flow over the cells and thus may participate in additional processes farther downstream. The importance of metabolic zonation is not yet fully understood; however, arguments have been made that it could increase metabolic efficiency and the ability of the liver to rapidly change its metabolic function (89). One case where sequential metabolism is well understood is that of ammonia detoxification by the liver, which was thoroughly investigated over 25 years ago (90). Here, blood entering the liver releases ammonia due to high glutaminase activity in the periportal region, and the increased free ammonia levels drive the urea cycle to convert ammonia into urea; however, due to the high K_m of CPS1, ammonia levels cannot be decreased to safe levels. Therefore, the last few perivenous hepatocytes in the acinus express glutamine synthetase, which has a high affinity for ammonia, thus enabling it to strip ammonia down to very low levels before blood leaves the liver. More recent studies suggest that these pathways may be under circadian control, which means that the metabolic picture of zonation may change depending on the simulated time of day or night. Thus, liver-on-chip systems may be well suited to dissect these complex dynamics.

A critical parameter in liver zonation is the oxygen tension gradient between the periportal inlet and perivenous outlet. It has been hypothesized that controlling this gradient alone can recreate many aspects of liver zonation in vivo (92). The oxygen gradient is usually borne out of the oxygen consumed by the cells in the device and can be predicted using computational fluid dynamic transport-reaction models; however, because of variability in cell seeding and metabolic activity in the device, flow rate (and likely other operating parameters) must be adjusted for each device and over time. Accomplishing this requires accurate in situ oxygen measurements. For example, Moya et al. (93) proposed oxygen monitoring using inkjet-printed amperometric sensors integrated into an ultrathin and porous membrane separating the cells from the flow channel and that allowed real-time monitoring of oxygen along the flow channel. Dynamic responses were easily measured, and the readouts were validated using conventional oxygen probes.

Li et al. (94) used microbeads made of polystyrene loaded with beads containing a palladium porphyrin, whose fluorescence intensity decreases due to quenching as local oxygen tension increases. The beads were directly infused into the system and became entrapped in the cell layer; thus, this approach is not specific to a particular device geometry and may potentially be used in a variety of configurations. In addition, fluorescent beads that are not sensitive to pO_2 were similarly infused, thus providing an internal standard. By taking the ratio of the pO_2-sensitive signal to that of the pO_2-independent signal, the authors could quantitatively measure oxygen tension around the cells in different locations of the system. More specifically, they could monitor and maintain a gradient between 18% O_2 at the inlet and 4% O_2 at the outlet, which resulted in clearly favored mitochondrial activity at the inlet and higher intracellular lipid accumulation near the outlet.

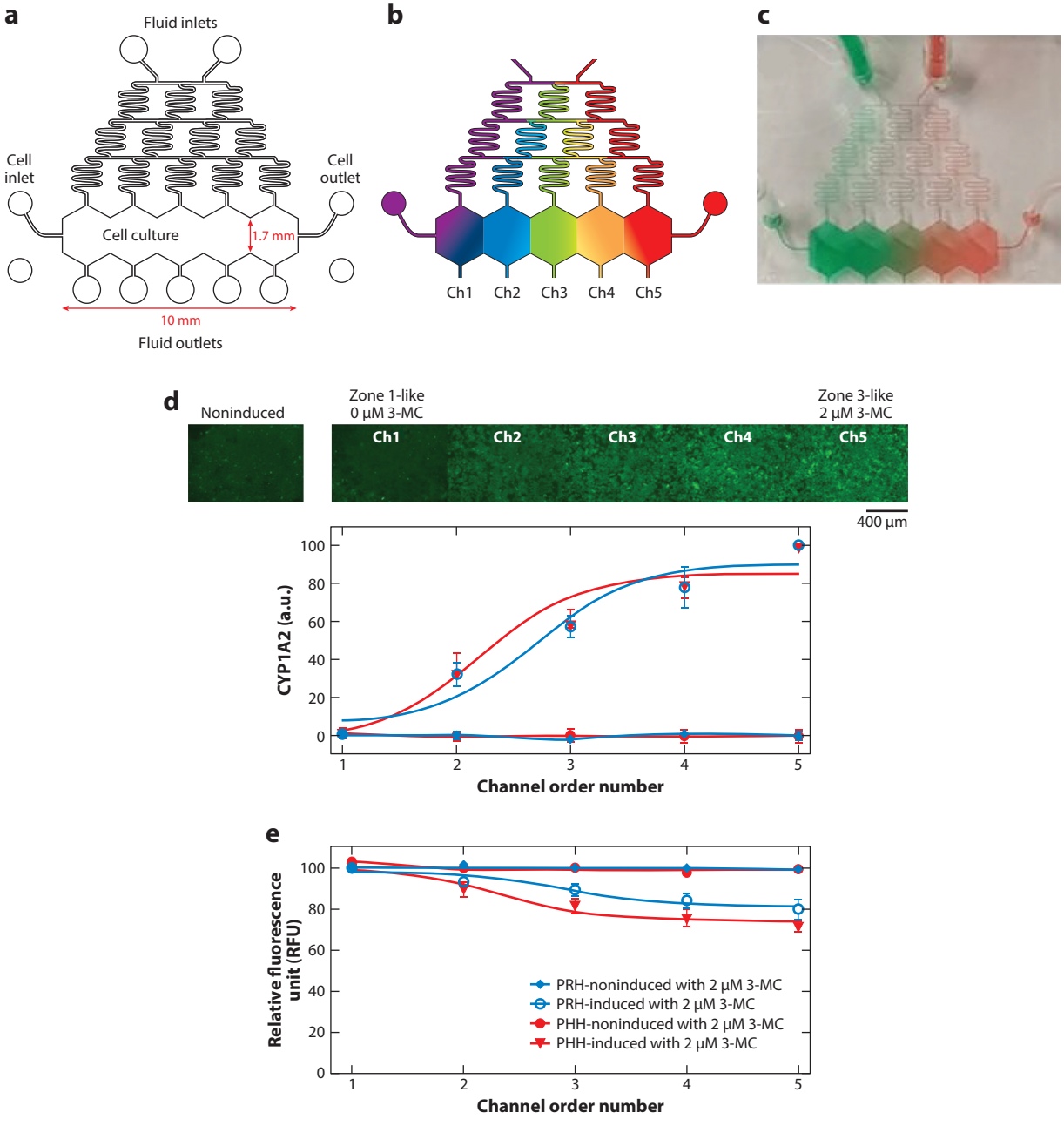

Figure 7

Recreating liver metabolic functions on a chip. Exposing liver cells in microfluidic devices to a concentration gradient of metabolites. (*a–c*) The cell culture chamber is uniformly seeded with liver cells, and then the culture chamber is arbitrarily divided into 5 different regions/channels where cells are exposed to different concentrations of metabolites. The different metabolite concentrations are generated via a microfluidic gradient generator. (*d*) For example, using a gradient of 3-MC, one can create spatial zonation of CYP1A2 activity. (*e*) This, in turn, causes zonation of acetaminophen toxicity, as evidenced by the decrease in viable stain fluorescence in channels pre-exposed to higher levels of 3-MC. Abbreviations: 3-MC, 3-methylcholanthrene; CYP, cytochrome P450; PHH, primary human hepatocyte; PRH, primary rat hepatocyte. Figure adapted with permission from Reference 91 under the terms of the Creative Commons Attribution (CC BY) License, **http://creativecommons.org/licenses/by/4.0**.

Another interesting feature of this system, which also incorporated three nonparenchymal cell types of the liver, was the preferential activation of stellate cells into myofibroblasts—the cell type involved in fibrosis—in the perivenous region of the device.

3.5. Endocrine Systems: Dynamic Responses and Effect of Biological Rhythms

Endocrine systems exhibit rapidly changing secretion profiles in response to stimuli. For example, pancreatic islets, one of the most thoroughly studied endocrine systems, change insulin secretion within minutes of exposure to a glucose stimulus; furthermore, the insulin release is biphasic and pulsatile in nature and under the control of intracellular calcium oscillations (95). The impact of islet size and cellular composition and the role of paracrine and autocrine signaling loops within islets on the secretion of insulin and other products, such as glucagon, pancreatic polypeptide, and somatostatin, can be studied in microfluidic systems. The ability to characterize the dynamics of protein secretion from tissue-engineered or freshly isolated pancreatic islets is also a promising avenue for standardizing islet transplantation procedures.

Dynamic measurement of small molecules, such as ions responsible for pH change, was pioneered by the development of the microphysiometer biosensor, which can monitor cellular responses over a timescale of seconds when cells are stimulated by different agonists (96). A limitation of the system is that it measures relatively nonspecific responses (e.g., the extracellular acidification rate) as surrogates for the biological event of interest (e.g., the secretion of insulin). Rapidly changing protein secretion profiles are challenging to monitor because they require methods that are specific, sensitive, and dynamically responsive. Immunoassays, one of the most common methods to measure a wide range of secreted products, are not inherently dynamically responsive because antibody–antigen binding is difficult to reverse without damaging the antibody itself. One workaround is to mix the effluent from the cultured islet cells with a continuous stream of fluorescently labeled immunoassay reagents and perform capillary electrophoresis to separate out the bound antigen–antibody complexes in the fluid phase; change in fluorescence intensity can then be correlated with insulin concentration. Dishinger et al. (97) successfully integrated on the same chip a microfluidic perfusion system for the pancreatic islets; together with the capillary electrophoresis separation and the fluorescence detection methods, this enabled measurements on a timescale of seconds for multiple islets simultaneously. Other common methods for detecting insulin in particular include electrochemical techniques, which use electrodes coated with catalysts to improve sensing performance and/or aptamers or molecular imprinted polymers to bind insulin in the solution (98). Aptamers are more resilient than antibodies, and it is possible to recycle them relatively easily and thus make them amenable to repeated measurements (99). Measuring multiple outputs may be possible through the use of several recognition elements targeted to different molecules. Raman spectroscopic analysis of engineered human islets, or their effluent, has also been reported as a plausible method to simultaneously characterize a variety of parameter changes in response to glucose stimulation (100). Time-resolved metabolomic analyses of cells on chip, which involve drawing samples from the chip into a mass spectrometer, have also been reported (101). In some cases, extracellular enzyme activities (such as lactate dehydrogenase or alanine aminotransferase) can be used as an indicator of cellular damage and can be inferred based on the detection of small molecules that are products of reactions catalyzed by the enzyme (102).

In recent years, there has been considerable interest in deciphering the role of biological rhythms on pathophysiology. Circadian behavior in rodents commonly used for drug testing is typically nocturnal and thus fundamentally different from diurnal species such as humans. Yet circadian rhythms are only beginning to be incorporated into microphysiological systems. The central hypothalamic clock is entrained by the 24-h day-night cycle in whole organisms, which, in

turn, modulates hormone levels that drive the peripheral clocks in individual tissues (103). Cyclical variation of endocrine hormones from various glands in the body play a major role in driving circadian liver physiology (104). Although it is not practical or even currently possible to integrate all of these tissue components on the same chip, infusing a medium containing time-varying levels of hormones that recreate the circadian environment on the chip may be possible. Studies that investigate other important biological cycles, notably the menstrual cycle, have also been reported (105).

Researchers have also begun exploring the interactions between endocrine glands and target tissues in microphysiological systems. For example, cocultures of pancreatic islets and liver spheroids were performed to create a feedback loop between glucose-stimulated secretion of insulin by the islets and insulin-stimulated glucose uptake by the liver spheroids (106). The results demonstrated cross talk between the two tissues and evolution toward a homeostatic state maintaining physiological levels of glucose. Furthermore, the system could emulate glucose tolerance tests that are used in humans. Incorporating daily medium changes, each time causing a peak in glucose levels, might be a simple way to incorporate circadian behavior into this system and better mimic daily variations in human metabolism (107). Incorporating real-time sensing of the relevant parameters would significantly improve our ability to characterize and understand the dynamic nature of such systems.

3.6. Electrical Functions

The reproduction of electrical functions in cardiac and neuronal tissues is among the first functions to have been recapitulated in vitro. It has received great attention for more than a decade and been presented in several recent reviews to which the reader is referred (108–110).

4. CONCLUSION: FUTURE CHALLENGES AND OPPORTUNITIES

The potential of OOC technology to become a competitive tool for assessing toxicity and therapeutic efficacy is being increasingly recognized in drug development (111, 112) and precision medicine (113) by researchers in academia, the pharmaceutical industry, and regulatory agencies (114). However, a number of challenges still need to be overcome for this technology to become fully integrated into the drug screening process and for further use in the clinic for precision medicine applications. One challenge is the need of biologically relevant cells that express their native phenotype. Given the heterogeneity of patients' cells and the scarcity of the material obtained from patients, iPSCs and tissue-specific progenitor cells that can be isolated and expanded in culture will certainly have a central role to play. iPSCs will allow the creation of patient-specific cells to be integrated into OOCs to create patient-on-chip systems to screen available drugs or combinations of drugs and tailor treatment plans to individual patients. However, for some pathologies for which the treatment should start immediately after diagnosis, such as sepsis, the establishment of the patient-on-chip model may take too long. Indeed, the lengthy differentiation process of iPSCs in fully functional adult cells is a major stumbling block in the stem cell field. Although the ability of OOCs to mimic complex tissue pathophysiology is clearly advantageous, it is also important to include simplicity and practicality as design criteria for OOC systems. Overly complex systems will not be adopted due to cost and difficulty of operation; thus, the simplest possible OOC should be designed that can address the question. The challenge of the physiological medium—that is currently highly tissue specific—required in multitissue systems will also have to be addressed, possibly by using a more physiological medium (115).

Notwithstanding these challenges, the opportunities created by OOCs are almost infinite. Although the field is still in its infancy, the number of tissue models, as shown in this review, that

already provide clinically relevant information keeps increasing. We expect that in the near future, more OOC models will be developed to focus on specific pathologies with their own biological questions. We also foresee that new clinically relevant readouts will be developed that can quantify increasingly complex in vivo–like biological events. Complex tissues and combinations of tissues, in multiorgan-on-chip systems, will also be developed, which will enable the assessment of both on-target effects and side effects of investigational therapeutics. New readout systems for OOCs are being developed (116, 117), and other noninvasive imaging methods commonly used in the clinic, such as optical coherence tomography, ultrasound, and nuclear magnetic resonance, to name a few, may also be used. The technological advances of OOCs, together with advances in patient-specific cell harvesting and differentiation using iPSC technology, will open new avenues for precision medicine applications, for example, by predicting patient-specific drug responses. Future companion diagnostic tools will also emerge from disease models set up in OOCs that will provide clinicians a decision-making tool to best tailor each patient's therapy.

DISCLOSURE STATEMENT

O. Guenat and T. Geiser are shareholders of the start-up AlveoliX AG, which aims at bringing organs-on-chip to market.

ACKNOWLEDGMENTS

O. Guenat thanks the Swiss Science National Foundation (project number 185365) for the generous support. F. Berthiaume's sabbatical stay in Dr. Guenat's laboratory was partially funded by the Burroughs Wellcome Fund. Anne Morbach is kindly acknowledged for the illustration of **Figure 2**.

LITERATURE CITED

1. Bhatia SN, Ingber DE. 2014. Microfluidic organs-on-chips. *Nat. Biotechnol.* 32(8):760–72
2. Marx U, Andersson TB, Bahinski A, Beilmann M, Beken S, et al. 2016. Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. *ALTEX* 33(3):272–321
3. Wang YI, Carmona C, Hickman JJ, Shuler ML. 2018. Multiorgan microphysiological systems for drug development: strategies, advances, and challenges. *Adv. Healthc. Mater.* 7(2):1701000
4. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. 2010. Reconstituting organ-level lung functions on a chip. *Science* 328(5986):1662–68
5. Bichsel CA, Hall SRR, Schmid RA, Guenat OT, Geiser T. 2015. Primary human lung pericytes support and stabilize *in vitro* perfusable microvessels. *Tissue Eng. A* 21(15–16):2166–76
6. Barrile R, Van Der Meer AD, Park H, Fraser JP, Simic D, et al. 2018. Organ-on-chip recapitulates thrombosis induced by an anti-CD154 monoclonal antibody: translational potential of advanced microengineered systems. *Clin. Pharmacol. Ther.* 104(6):1240–48
7. Sung JH, Shuler ML. 2009. A micro cell culture analog (microCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. *Lab Chip* 9(10):1385–94
8. Behjati S, Huch M, van Boxtel R, Karthaus W, Wedge DC, et al. 2014. Genome sequencing of normal cells reveals developmental lineages and mutational processes. *Nature* 513:422–25
9. Shi Y, Inoue H, Wu JC, Yamanaka S. 2016. Induced pluripotent stem cell technology: a decade of progress. *Nat. Rev.* 16(2):115–30
10. Williams LA, Davis-Dusenbery BN, Eggan KC. 2012. SnapShot: directed differentiation of pluripotent stem cells. *Cell* 149:1174.e1
11. Price PJ. 2017. Best practices for media selection for mammalian cells. *In Vitro Cell. Dev. Biol.* 53:673–81
12. Gauvin R, Mehmet R, Merryman WD, Khademhosseini A. 2012. Hydrogels and microtechnologies for engineering the cellular microenvironment. *Wires Nanomed. Nanobiotechnol.* 4(3):235–46

13. da Silveira dos Santos AX, Liberali P. 2019. From single cells to tissue self-organization. *FEBS J.* 286:1495–513
14. Wang Y, Ahmad AA, Sims CE, Magness ST, Allbritton NL. 2014. In vitro generation of colonic epithelium from primary cells guided by microstructures. *Lab Chip* 14(9):1622–31
15. Sieber S, Wirth L, Cavak N, Koenigsmark M, Marx U, et al. 2018. Bone marrow-on-a-chip: Long-term culture of human haematopoietic stem cells in a three-dimensional microfluidic environment. *J. Tissue Eng. Regen. Med.* 12:479–89
16. Zamprogno P, Wüthrich S, Achenbach S, Stucki JD, Hobi N, et al. 2019. Second-generation lung-on-a-chip array with a stretchable biological membrane. bioRxiv 608919. **https://doi.org/10.1101/608919**
17. Zhang B, Montgomery M, Chamberlain MD, Ogawa S, Korolj A, et al. 2016. Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis. *Nat. Mater.* 15(6):669–78
18. Sano E, Mori C, Nashimoto Y, Yokokawa R, Kotera H, Torisawa Y. 2018. Engineering of vascularized 3D cell constructs to model cellular interactions through a vascular network. *Biomicrofluidics* 12:042204
19. Homan KA, Gupta N, Kroll KT, Kolesky DB, Skylar-Scott M, et al. 2019. Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nat. Methods* 16:255–62
20. Schutgens F, Rookmaaker MB, Margaritis T, Rios A, Ammerlaan C, et al. 2019. Tubuloids derived from human adult kidney and urine for personalized disease modeling. *Nat. Biotechnol.* 37:303–13
21. Park SE, Georgescu A, Huh D. 2019. Organoids-on-a-chip. *Science* 364:960–65
22. Takebe T, Zhang B, Radisic M. 2017. Synergistic engineering: organoids meet organs-on-a-chip. *Cell Stem Cell* 21(3):297–300
23. David L, Dumitrascu DL. 2017. The bicentennial of the stethoscope: a reappraisal. *Clujul Med.* 90(3):361–63
24. Huh D, Fujioka H, Tung Y-C, Futai N, Paine R, et al. 2007. Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. *PNAS* 104(48):18886–91
25. Edlow BL, Mareyam A, Horn A, Polimeni JR, Dylan M. 2019. 7 Tesla MRI of the *ex vivo* human brain at 100 micron resolution. bioRxiv 649822. **https://doi.org/10.1101/649822**
26. Bailey KE, Floren ML, Ovidio TJD, Lammers SR, Stenmark KR, Magin XCM. 2019. Tissue-informed engineering strategies for modeling human pulmonary diseases. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 316:L303–20
27. Cai Y, Schrenk S, Goines J, Davis GE, Boscolo E. 2019. Constitutive active mutant TIE2 induces enlarged vascular lumen formation with loss of apico-basal polarity and pericyte recruitment. *Sci. Rep.* 9:12352
28. Haase K, Kamm RD. 2017. Advances in on-chip vascularization. *Regen. Med.* 12(3):285–302
29. Zhao H, Chappell JC. 2019. Microvascular bioengineering: a focus on pericytes. *J. Biol. Eng.* 13:26
30. Soleimani S, Shamsi M, Ghazani AM, Modarres H, Valente K, et al. 2018. Translational models of tumor angiogenesis: a nexus of in silico and in vitro models. *Biotechnol. Adv.* 36:880–93
31. Ko J, Ahn J, Kim S, Lee Y, Lee J, et al. 2019. Tumor spheroid-on-a-chip: a standardized microfluidic culture platform for investigating tumor angiogenesis. *Lab Chip* 19:2822–33
32. Ko J, Lee Y, Lee S, Lee S, Jeon NL. 2019. Human ocular angiogenesis-inspired vascular models on an injection-molded microfluidic chip. *Adv. Healthc. Mater.* 8:1900328
33. Huh D, Leslie DC, Matthews BD, Fraser JP, Jurek S, et al. 2012. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. *Sci. Transl. Med.* 4:159ra147
34. Kim DW, Zloza A, Broucek J, Schenkel JM, Ruby C, et al. 2014. Interleukin-2 alters distribution of CD144 (VE-cadherin) in endothelial cells. *J. Transl. Med.* 12:113
35. Jain A, Barrile R, van der Meer AD, Mammoto A, Mammoto T, et al. 2017. A primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. *Clin. Pharmacol. Ther.* 103(2):332–40
36. Zeinali S, Bichsel CA, Hobi N, Funke M, Marti TM, et al. 2018. Human microvasculature-on-a chip: anti-neovasculogenic effect of nintedanib in vitro. *Angiogenesis* 21(4):861–71
37. Bichsel CA, Wang L, Froment L, Berezowska S, Müller S, et al. 2017. Increased PD-L1 expression and IL-6 secretion characterize human lung tumor-derived perivascular-like cells that promote vascular leakage in a perfusable microvasculature model. *Sci. Rep.* 7:10636

38. Jiménez-Torres JA, Virumbrales-Muñoz M, Sung KE, Hee M, Abel EJ, Beebe DJ. 2019. Patient-specific organotypic blood vessels as an in vitro model for anti-angiogenic drug response testing in renal cell carcinoma. *EBioMedicine* 42:408–19
39. Garcia AN, Vogel SM, Komarova YA, Malik AB. 2011. Permeability of endothelial barrier: cell culture and *in vivo* models. *Methods Mol. Biol.* 763:333–54
40. van der Helm MW, Odijk M, Frimat J, van der Meer AD, Eijkel JCT, et al. 2016. Direct quantification of transendothelial electrical resistance in organs-on-chips. *Biosens. Bioelectron.* 85:924–29
41. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. 2015. TEER measurement techniques for in vitro barrier model systems. *J. Lab. Autom.* 20(2):107–26
42. Offeddu GS, Haase K, Gillrie MR, Li R, Morozova O, et al. 2019. An on-chip model of protein paracellular and transcellular permeability in the microcirculation. *Biomaterials* 212:115–25
43. Schnoor M. 2015. Endothelial actin-binding proteins and actin dynamics in leukocyte transendothelial migration. *J. Immunol.* 194:3535–41
44. Strilic B, Offermanns S. 2017. Intravascular survival and extravasation of tumor cells. *Cancer Cell* 32:282–93
45. Tang Y, Soroush F, Sun S, Liverani E, Langston JC, et al. 2018. Protein kinase C-delta inhibition protects blood-brain barrier from sepsis-induced vascular damage. *J. Neuroinflamm.* 15:309
46. Kim JJ, Ellett F, Thomas CN, Jalali F, Anderson RR, et al. 2019. A microscale, full-thickness, human skin on a chip assay simulating neutrophil responses to skin infection and antibiotic treatments. *Lab Chip* 19:3094–103
47. Menon NV, Tay HM, Pang KT, Dalan R, Wong SC, et al. 2018. A tunable microfluidic 3D stenosis model to study leukocyte-endothelial interactions in atherosclerosis. *APL Bioeng.* 2:016103
48. Bersini S, Miermont A, Pavesi A, Kamm RD, Thiery P, et al. 2018. A combined microfluidic-transcriptomic approach to characterize the extravasation potential of cancer cells. *Oncotarget* 9(90):36110–25
49. Boussommier-Calleja A, Atiyas Y, Haase K, Headley M, Lewis C, Kamm RD. 2019. The effects of monocytes on tumor cell extravasation in a 3D vascularized microfluidic model. *Biomaterials* 198:180–93
50. Schwartz LH, Litière S, de Vries E, Ford R, Mandrekar S, et al. 2016. RECIST 1.1 update and clarification: from the RECIST Committee. *Eur. J. Cancer* 62:132–37
51. Hodi FS, Ballinger M, Lyons B, Soria JC, Nishino M, et al. 2018. Immune-modified response evaluation criteria in solid tumors (imRECIST): refining guidelines to assess the clinical benefit of cancer immunotherapy. *J. Clin. Oncol.* 36:850–58
52. Fidler IJ, Kripke ML. 2015. The challenge of targeting metastasis. *Cancer Metastasis Rev.* 34:635–41
53. Mallat RK, John CM, Kendrick DJ, Braun A. 2017. The vascular endothelium: a regulator of arterial tone and interface for the immune system. *Clin. Rev. Clin. Lab. Sci.* 54(7–8):458–70
54. Günther A, Yasotharan S, Vagaon A, Lochovsky C, Pinto S, et al. 2010. A microfluidic platform for probing small artery structure and function. *Lab Chip* 10:2341–49
55. Ferguson-Myrthil N. 2012. Vasopressor use in adult patients. *Cardiol. Rev.* 20(3):153–58
56. Li Y, Wu Y, Liu Y, Deng Q, Mak M, Yang X. 2019. Atmospheric nanoparticles affect vascular function using a 3D human vascularized organotypic chip. *Nanoscale* 11:15537–49
57. Kim S, Takayama S. 2015. Organ-on-a-chip and the kidney. *Kidney Res. Clin. Pract.* 34(3):165–69
58. Wilmer MJ, Ng CP, Lanz HL, Vulto P, Suter-Dick L, Masereeuw R. 2016. Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. *Trends Biotechnol.* 34(2):156–70
59. Bajaj P, Chowdhury SK, Yucha R, Kelly EJ, Xiao G. 2018. Emerging kidney models to investigate metabolism, transport, and toxicity of drugs and xenobiotics. *Drug Metab. Dispos.* 46:1692–702
60. Nieskens TTG, Wilmer MJ. 2016. Kidney-on-a-chip technology for renal proximal tubule tissue reconstruction. *Eur. J. Pharmacol.* 790:46–56
61. Zhou M, Zhang X, Wen X, Wu T, Wang W. 2016. Development of a functional glomerulus at the organ level on a chip to mimic hypertensive nephropathy. *Sci. Rep.* 6:31771

62. Musah S, Mammoto A, Ferrante TC, Jeanty SSF, Mammoto T, et al. 2017. Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. *Nat. Biomed. Eng.* 1:0069
63. Vormann MK, Gijzen L, Hutter S, Boot L, Nicolas A, et al. 2018. Nephrotoxicity and kidney transport assessment on 3D perfused proximal tubules. *AAPS J.* 20:90
64. Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, Ramme AP, et al. 2015. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip* 15:2688–99
65. Bein A, Shin W, Jalili-Firoozinezhad S, Park MH, Sontheimer-Phelps A, et al. 2018. Microfluidic organ-on-a-chip models of human intestine. *Cell. Mol. Gastron. Hepatol.* 5(4):659–68
66. Wang Y, Kim R, Hinman SS, Zwarycz B, Magness ST, Allbritton NL. 2018. Bioengineered systems and designer matrices that recapitulate. *Cell. Mol. Gastron. Hepatol.* 5:440–53
67. Du Y, Khandekar G, Llewellyn J, Polacheck W, Chen C, Wells RG. 2019. A bile duct-on-a-chip with organ-level functions. *Hepatology*. **https://doi.org/10.1002/hep.30918**
68. Nelson CM, Gleghorn JP, Pang M, Jaslove JM, Goodwin K, et al. 2017. Microfluidic chest cavities reveal that transmural pressure controls the rate of lung development. *Development* 144:4328–35
69. Manfrin A, Tabata Y, Paquet ER, Vuaridel AR, Rivest FR, et al. 2019. Engineered signaling centers for the spatially controlled patterning of human pluripotent stem cells. *Nat. Methods* 16:640–48
70. Samal P, van Blitterswijk C, Truckenmüller R, Giselbrecht S. 2019. Grow with the flow: when morphogenesis meets microfluidics. *Adv. Mater.* 31:1805764
71. Chen CZC, Raghunath M. 2009. Focus on collagen: in vitro systems to study fibrogenesis and antifibrosis-state of the art. *Fibrogenesis Tissue Repair* 2:7
72. Xu X, Li Z, Cai L, Calve S, Neu CP. 2016. Mapping the nonreciprocal micromechanics of individual cells and the surrounding matrix within living tissues. *Sci. Rep.* 6:24272
73. Booth AJ, Hadley R, Cornett AM, Dreffs AA, Matthes SA, et al. 2012. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. *Am. J. Respir. Crit. Care Med.* 186(9):866–76
74. Knudsen L, Ruppert C, Ochs M. 2017. Tissue remodelling in pulmonary fibrosis. *Cell Tissue Res.* 367:607–26
75. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. 2008. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? *Int. J. Biochem. Cell Biol.* 40(3):362–82
76. Felder M, Trüeb B, Stucki AO, Borcard S, Daniel J. 2019. Impaired wound healing of alveolar lung epithelial cells in a breathing lung-on-a-chip. *Front. Bioeng. Biotechnol.* 7:3
77. Funke M, Geiser T. 2015. Idiopathic pulmonary fibrosis: the turning point is now! *Swiss Med. Wkly.* 145:w14139
78. Gabbiani G. 2003. The myofibroblast in wound healing and fibrocontractive diseases. *J. Pathol.* 200:500–3
79. Kong M, Lee J, Yazdi IK, Miri AK, Lin Y, et al. 2019. Cardiac fibrotic remodeling on a chip with dynamic mechanical stimulation. *Adv. Healthc. Mater.* 8:1801146
80. Asmani M, Velumani S, Li Y, Wawrzyniak N, Chen Z, et al. 2018. Fibrotic microtissue array to predict anti-fibrosis drug efficacy. *Nat. Commun.* 9:2066
81. Ebinuma H, Saito H, Komuta M, Ojiro K, Wakabayashi K, Usui S. 2011. Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: comparison with Fibroscan. *J. Gastroenterol.* 46:1238–48
82. Bulusu V, Prior N, Snaebjornsson M, Kuehne A, Sonnen K, et al. 2017. Spatiotemporal analysis of a glycolytic activity gradient linked to mouse embryo mesoderm development. *Dev. Cell* 40:331–41
83. Rinnerthaler M, Streubel MK, Bischof J, Richter K. 2015. Skin aging, gene expression and calcium. *Exp. Gerontol.* 68:59–65
84. Shin W, Hinojosa C, Ingber D, Kim HJ. 2019. Human intestinal morphogenesis controlled by transepithelial morphogen gradient and flow-dependent physical cues in a microengineered gut-on-a-chip. *iScience* 15:391–406

85. Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, Camacho DM, Fadel CW, et al. 2019. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat. Biomed. Eng.* 3:520–31
86. König M, Holzhütter HG, Berndt N. 2013. Metabolic gradients as key regulators in zonation of tumor energy metabolism: a tissue-scale model-based study. *Biotechnol. J.* 8:1058–69
87. Shin W, Wu A, Massidda MW, Foster C, Thomas N, et al. 2019. A robust longitudinal co-culture of obligate anaerobic gut microbiome with human intestinal epithelium in an anoxic-oxic interface-on-a-chip. *Front. Bioeng. Biotechnol.* 7:13
88. Soto-Gutierrez A, Gough A, Vernetti LA, Taylor DL, Monga SP. 2017. Pre-clinical and clinical investigations of metabolic zonation in liver diseases: the potential of microphysiology systems. *Exp. Biol. Med.* 242:1605–16
89. Gebhardt R, Matz-Soja M. 2014. Liver zonation: novel aspects of its regulation and its impact on homeostasis. *World J. Gastroenterol.* 20(26):8491–504
90. Häussinger D, Lames W, Moorman A. 1992. Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* 46(1–3):72–93
91. Kang YB, Eo J, Mert S, Yarmush M, Usta B, 2018, Metabolic patterning on a chip: towards in vitro liver zonation of primary rat and human hepatocytes. *Sci. Rep*. 8(1):8951
92. Lee-Montiel FT, George SM, Gough AH, Sharma AD, Wu J, et al. 2017. Control of oxygen tension recapitulates zone-specific functions in human liver microphysiology systems. *Exp. Biol. Med.* 242:1617–32
93. Moya A, Ortega-Ribera M, Guimera X, Sowade E, Zea M, et al. 2018. Online oxygen monitoring using integrated inkjet-printed sensors in a liver-on-a-chip system. *Lab Chip* 18:2023–35
94. Li X, George SM, Vernetti L, Gough AH, Taylor DL. 2018. A glass-based, continuously zonated and vascularized human liver acinus microphysiological system (vLAMPS) designed for experimental modeling of diseases and ADME/TOX. *Lab Chip* 18:2614–31
95. Castiello FR, Heileman K, Tabrizian M. 2016. Microfluidic perfusion systems for secretion fingerprint analysis of pancreatic islets: applications, challenges and opportunities. *Lab Chip* 16:409–31
96. McConnell H, Rice P, Wada G, Owicki J, Parce W. 1991. The microphysiometer biosensor. *Curr. Opin. Struct. Biol.* 1(4):647–52
97. Dishinger JF, Reid KR, Kennedy RT. 2009. Quantitative monitoring of insulin secretion from microfluidic chip. *Anal. Chem*. 81:3119–27
98. Gerasimov JY, Schaefer CS, Yang W, Grout RL, Lai RY. 2013. Development of an electrochemical insulin sensor based on the insulin-linked polymorphic region. *Biosens. Bioelectron*. 42:62–68
99. Lee J, So H, Jeon E. 2008. Aptamers as molecular recognition elements for electrical nanobiosensors. *Anal. Bioanal. Chem*. 390:1023–32
100. Zbinden A, Marzi J, Schlünder K, Probst C, Urbanczyk M, et al. 2019. Non-invasive marker-independent high content analysis of a microphysiological human pancreas-on-a-chip model. *Matrix Biol*. **https://doi.org/10.1016/j.matbio.2019.06.008**
101. Marasco CC, Enders JR, Seale KT, Mclean JA, Wikswo JP. 2015. Real-time cellular exometabolome analysis with a microfluidic-mass spectrometry platform. *PLOS ONE* 10(2):e0117685
102. La Cour JB, Generelli S, Barbe L, Guenat OT. 2016. Low-cost disposable ALT electrochemical microsensors for in-vitro hepatotoxic assessment. *Sens. Actuators B* 228:360–65
103. Bae S, Fang MZ, Rustgi V, Zarbl H, Androulakis I. 2019. At the interface of lifestyle, behavior, and circadian rhythms: metabolic implications. *Front. Nutr.* 28. **https://doi.org/10.3389/FNUT.2019.00132**
104. Cyr KJ, Avaldi OM, Wikswo JP. 2017. Circadian hormone control in a human-on-a-chip: in vitro biology's ignored component? *Exp. Biol. Med.* 242:1714–31
105. Xiao S, Coppeta JR, Rogers HB, Isenberg BC, Zhu J, et al. 2017. A microfluidic culture model of the reproductive tract and 28-day menstrual cycle. *Nat. Commun*. 8:14584
106. Bauer S, Wennbe C, Kanebratt KP, Durieux I, Andersson S, et al. 2017. Functional coupling of human pancreatic islets and liver spheroids on-a-chip: towards a novel human ex vivo type 2 diabetes model. *Sci. Rep.* 7:14620

107. Maass C, Dallas M, Labarge ME, Shockley M, Geishecker E, et al. 2018. Establishing quasi-steady state operations of microphysiological systems (MPS) using tissue-specific metabolic dependencies. *Sci. Rep.* 8:8015
108. Meyer T, Tiburcy M, Zimmermann W. 2019. Cardiac macrotissues-on-a-plate models for phenotypic drug screens. *Adv. Drug Deliv. Rev.* 140:93–100
109. Fantuzzo JA, Hart RP, Zahn JD, Pang ZP. 2019. Compartmentalized devices as tools for investigation of human brain network dynamics. *Dev. Dyn.* 248:65–77
110. Frimat J, Luttge R. 2019. The need for physiological micro-nanofluidic systems of the brain. *Front. Bioeng. Biotechnol.* **https://doi.org/10.3389/fbioe.2019.00100**
111. Esch EW, Bahniski A, Huh D. 2015. Organs-on-chips at the frontiers of drug discovery. *Nat. Rev. Drug Discov.* 14(4):248–60
112. Selimović Š, Dokmeci MR, Khademhosseini A. 2013. Organs-on-a-chip for drug discovery. *Curr. Opin. Pharmacol.* 13(5):829–33
113. Ronaldson-Bouchard K, Vunjak-Novakovic G. 2018. Organs-on-a-chip: a fast track for engineered human tissues in drug development. *Cell Stem Cell* 22(3):310–24
114. Fabre KM, Livingston C, Tagle DA. 2014. Organs-on-chips (microphysiological systems): tools to expedite efficacy and toxicity testing in human tissue. *Exp. Biol. Med.* 239(9):1073–77
115. Ackermann T, Tardito S. 2019. Cell culture medium formulation and its implications in cancer metabolism. *Trends Cancer* 5(6):329–32
116. Peel S, Corrigan A, Ehrhardt B, Jang K, Caetano-Pinto P, et al. 2019. Introducing an automated high content confocal imaging approach for Organs-on-Chips. *Lab Chip* 19:410–21
117. Polat A, Hassan S, Yildirim I, Oliver LE, Mostafaei M, et al. 2019. A miniaturized optical tomography platform for volumetric imaging of engineered living systems. *Lab Chip* 19:550–61