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CRITICAL REVIEW

Organs-on-chips: breaking the *in vitro* impasse

Andries D. van der Meer and Albert van den Berg

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In vitro models of biological tissues are indispensable tools for unraveling human physiology and pathogenesis. They usually consist of a single layer of a single cell type, which makes them robust and suitable for parallelized research. However, due to their simplicity, *in vitro* models are also less valid as true reflections of the complex biological tissues of the human body. Even though the realism of the models can be increased by including more cell types, this will inevitably lead to a decrease in robustness and throughput. The constant trade-off between realism and simplicity has led to an impasse in the development of new *in vitro* models. Organs-on-chips, a class of microengineered *in vitro* tissue models, have the potential to break the *in vitro* impasse. These models combine an artificially engineered, physiologically realistic cell culture microenvironment with the potential for parallelization and increased throughput. They are robust, because the engineered physiological, organ-level features such as tissue organization, geometry, soluble gradients and mechanical stimulation are well-defined and controlled. Moreover, their microfluidic properties and integrated sensors pave the way for high-throughput studies. In this review, we define the *in vitro* impasse, we explain why organs-on-chips have the potential to break the impasse and we formulate a view on the future of the field. We focus on the design philosophy of organs-on-chips, the integration of technology and biology and on how to connect to the potential end-users.

Physiology *in vivo* and *in vitro*

Human physiology is the science of the function of human bodies and their organ systems. Needless to say, human physiology is of great importance for understanding bodily dysfunction and pathogenesis and is therefore very much connected to the fields of medicine, drug development and toxicology. *In vivo* experimentation, in which the bodies of humans or model organisms as a whole are observed and manipulated, is the most relevant and direct method to study human physiology.

BIOS/Lab on a Chip group, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands.
E-mail: a.d.vandermeer@utwente.nl, a.vandenberg@utwente.nl

Bodily functions are emergent phenomena that depend on the interaction and adaptation of many lower-level components, such as tissues, cells, proteins and genes. Therefore, it can be difficult to unravel the underlying mechanisms of many physiological phenomena solely by performing *in vivo* studies. Moreover, *in vivo* testing is inherently low-throughput. This is a problem in drug development and toxicology, in which the physiological effects of thousands of compounds need to be evaluated.

Because of the limitations of *in vivo* testing, biologists have adopted a reductionist approach. They isolate a limited number of cells and study these in a well-defined laboratory setting. These *in vitro* tissue models have the distinct advantage that measurements on them are relatively robust, predictable and repeatable. Moreover, *in vitro* tissue models are fast, high-throughput, simple, can be thoroughly analyzed and can be set up with healthy,

Insight, innovation, integration

Human physiology is a complex phenomenon. Unraveling the mechanisms by which physiological complexity arises is no easy matter. Therefore, biologists perform some of their research in simple *in vitro* models, in which the isolated cells or tissues of interest are cultured and analyzed in the laboratory. The physiological realism of such isolated *in vitro* models can potentially be increased by optimizing the culture microenvironment in terms of fluid flow, mechanical stretch, confinement, *etc*. Organs-on-chips are microengineered platforms that integrate engineered, physiologically relevant microenvironments and cultured biological tissue. By this integration, a new class of *in vitro* models appears that manages to combine physiological realism with well-controlled, simple culture conditions. Therefore, organs-on-chips can potentially have a big impact on physiological research.

modified or diseased human tissue. Therefore, *in vitro* models are indispensable tools to complement the complex results of *in vivo* studies.[1–3]

The *in vitro* impasse: realism *versus* simplicity

Paradoxically, the main advantage of *in vitro* model systems is also their main disadvantage. *In vitro* models are simple, but this simplicity also means that they fail to mimic key aspects of the human body. This failure to reproduce physiologically relevant factors can cause skewed results, misinterpretations and false conclusions.[4,5] However, when increasing the realism of the system by including more cell types, the model becomes more complex: the system consists of more 'parts', each of which harbors uncertain behavior, and each of which interacts with the other parts in an unpredictable way.[6] As a result, the behavior of the model as a whole becomes more unpredictable and more prone to variations based on slight differences in the starting situation. This unpredictability of the model leads to less robust measurements (Fig. 1). Because of the resultant trade-off between realism and simplicity, the field of *in vitro* tissue models has arrived at an impasse. A good illustration of the lack of innovation in the field is the fact that the most widely used *in vitro* models are still simple layers of cells cultured on a polystyrene surface or on a semi-permeable plastic membrane.

Breaking the impasse: microenvironment engineering

Despite the lack of widespread innovation, there is a clear sense of direction in the field as to how the *in vitro* impasse can be broken. Numerous researchers have argued—and shown—that more realistic models can be generated by artificially engineering key aspects of the *in vivo* microenvironment of cells and tissues.[7–9] Such *in vitro* engineering of the cell culture microenvironment can induce self-organizing, realistic behavior in healthy cells and cancer cells alike. Interestingly, because microenvironment engineering is essentially nothing more than an alteration of the culture conditions, it does not increase the biological complexity and unpredictability of the model in the way that the addition of more cell types would do. In other words, models that employ the approach of microenvironment engineering have the potential to break the realism-*versus*-simplicity impasse.

One of the challenges in the development of such micro-engineered models is to find appropriate aspects of the *in vivo* microenvironment that can be mimicked *in vitro* by engineering. So far, numerous geometrical, mechanical and biochemical factors have already been identified that steer cell lines, primary cells, stem cells and cancer cells towards a more realistic phenotype.[10–17] For example, growing stem cells on substrates of various stiffness induces differentiation of these cells into different lineages.[11] Or, growing endothelial cells on a three-dimensional matrix induces the formation of vascular tubules instead of flat cell monolayers.[18] Subjecting hematopoietic progenitor cells to stable gradients of cytokines induces homing of these cells to artificial bone marrow compartments.[19] Such examples illustrate the strong synergy between engineering of the culture environment and the self-organizational capacity of cells. Even simple factors like the stiffness of a substrate or the presence of a gradient can induce a cascade of self-organizational events in cell populations that lead to a more realistic model.

Still, even though a lot of important factors from the microenvironment that can be artificially engineered *in vitro* have been identified, a huge challenge remains. Namely, how to design and build *in vitro* systems that generate these microenvironmental factors in a straightforward, high-throughput and reproducible manner. This is the challenge

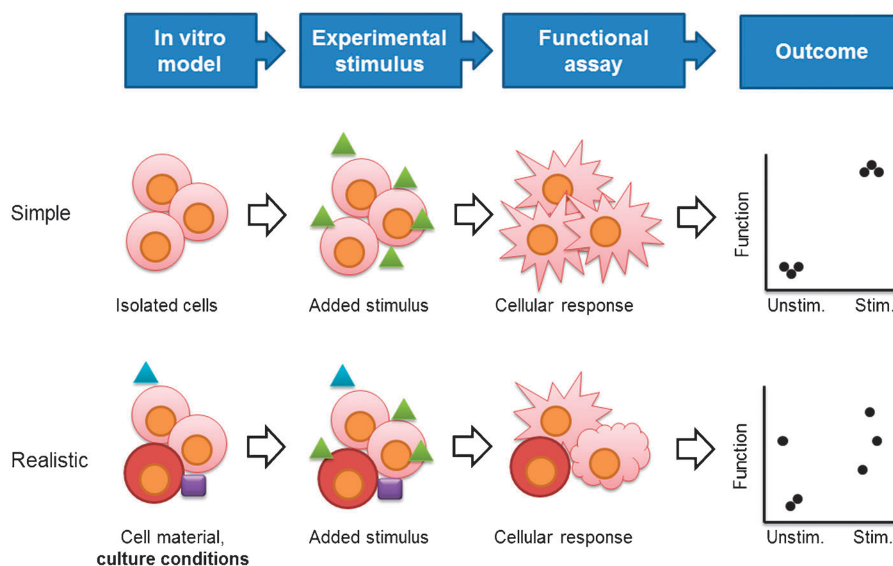

Fig. 1 Simple *versus* realistic *in vitro* models. The tissue of an *in vitro* model is subjected to an experimental stimulus, which is expected to elicit a functional response. By analyzing this response, the effect of the stimulus can be plotted. In a simple, isolated system (top), the response is uniform and predictable, but may not reflect the real-life response. In a more realistic model with more complex culture conditions and additional cell material (bottom), the response to the stimulus becomes blurred and unpredictable. When developing new *in vitro* models, the challenge is to include physiologically relevant culture conditions without affecting the robustness of the cellular response to the experimental stimulus.

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that is currently being addressed by new, integrated, micro-engineered *in vitro* platforms: organs-on-chips.[20]

Organs-on-chips: platforms for microenvironment engineering

Organs-on-chips are a class of microdevices for *in vitro* tissue culture that feature a physiologically relevant, engineered micro-environment. The devices are termed 'chips' because the design principles are based on microchip technology, featuring multiple, controllable parallel channels, splitting and merging channels, pumps, valves and integrated electrical and biochemical sensors. The devices are termed 'organs', because some of the engineered microenvironmental stimuli are derived from organ-level functions, such as breathing-derived mechanical stretch for lung cells,[21] fluid shear stress for vascular cells[22] and cyclic physical loading for cardiomyocytes.[23]

Organ-on-a-chip-technology is rooted in the fields of biological microelectromechanical systems (bioMEMS),[24] microfluidics[25] and biomimetics.[26] BioMEMS deals with microengineered systems in biological contexts, microfluidics is about the behavior and control of fluids in such systems and biomimetics revolves around the artificial engineering of functions that are found in biological systems. From these roots, it follows that the research on organs-on-chips focuses strongly on engineering and technology. In the recent years, the role of cell biology and, more specifically, the self-organizing capacity of cells to form functional tissues is starting to play a more pronounced role in the field, as well. An important consideration in this context is to decide what source of cells should be used to design organs-on-chips. Immortalized cell lines, primary cell material, stem cells or induced pluripotent stem cells all have their pros and cons when they are applied to develop these new *in vitro* models.[20] Human cell lines are a widely available, well-established source of cell material. The main disadvantages of using these cells are their slight phenotypic mismatches with *in vivo* tissues and the fact that they are genetically homogenous and not patient-specific. Primary cell material does not suffer from these disadvantages. However, it is difficult to obtain and to maintain in culture for longer periods of time. By using stem cells or by generating induced pluripotent stem cells from patient material, the difficulties of availability and culturing are reduced. However, steering stem cells towards a well-differentiated state and maintaining this differentiated phenotype can be difficult. Because none of the cell sources are completely superior to the others, the use of cells from a certain source will usually depend on the specific application.

The general approach for developing an organ-on-a-chip is to identify key aspects of the geometrical, mechanical and biochemical microenvironment of the tissue of interest. Some of these aspects will be local, like the excretion of biochemical factors by neighboring cells and tissues. Other aspects will be derived from higher organizational levels, like the mechanical stretching of an entire organ. Once one or several key aspects of the physiological microenvironment of the tissue of interest have been identified, a microengineering approach is applied to introduce these key aspects in the tissue model and bring them under complete control of the researcher. Then, isolated cells are introduced in the model and subjected to the engineered stimuli. By biologically responding to the stimuli, the cells self-organize into tissue that displays more realistic functionality than tissue that has been cultured in conventional *in vitro* systems. Finally, functional output parameters of the cultured tissue are identified and measured. Most organs-on-chips are flat and optically accessible, and can therefore be easily studied by microscopy to identify morphological output parameters. Moreover, some organs-on-chips already have integrated technology for measuring electrochemical or fluorescent tissue parameters.

To sum up, organs-on-chips include key aspects of the tissue microenvironment, without decreasing the robustness of measurements on the system. This robust realism is due to implementation of microenvironmental parameters by engineering, which in turn allows for a high level of spatiotemporal control over the culture conditions. Moreover, the models have dimensions in the micrometre range, so the amounts of cells, drugs and culture media that are needed for an experiment are small. This allows for parallelization and increased throughput. All these advantages make organs-on-chips ideal engineering platforms to realize new, realistic *in vitro* tissue models that are based on the philosophy of microenvironment engineering.

To illustrate the design philosophy behind organs-on-chips, we will discuss a number of the models in more detail (Fig. 2).

Lung-on-a-chip

The elementary tissue unit of the lung is the layer of epithelial and endothelial cells over which the exchange of gas between air and blood takes place. The geometry of the epithelial–endothelial interface is important, with the epithelium facing the air and the endothelium facing the blood. Moreover, the cells are subjected to distinct mechanical forces. For example, the lung tissue stretches with each respiratory cycle and in certain diseases the epithelial monolayer undergoes damaging shear stresses that are caused by movement of fluid trapped in the lungs. It is well-known that mechanical properties of the tissue microenvironment are physiologically important, both in lung tissue and other tissues.[27,28]

The geometry of the lung epithelial–endothelial interface is relatively easy to reproduce *in vitro*. A lot of models exist in which epithelial and endothelial cells are grown on either side of a thin porous membrane.[29] However, it proved to be a lot more difficult to include the mechanical microenvironment in simple tissue models of the lung.

In recent years, microfluidic models have appeared that allow precise treatment of an airway epithelial monolayer with well-defined liquid plug shear stresses,[30,31] mechanical stretch,[21] or both.[32] Liquid plugs are introduced by pumping fluid into the microfluidic culture channels or by applying negative pressures to partially fluid-filled channels. Mechanical stretch is established by culturing cells on a flexible membrane that can be deformed by applying negative pressures to adjacent control chambers. Because of the inclusion of a membrane, epithelial and endothelial cells can be co-cultured in one device, exposing one monolayer to air and the other to culture medium. A clear proof of the usefulness of mimicking such physiological aspects in a culture model was provided by Huh *et al.* in a seminal paper for the field of organs-on-chips. They demonstrated that nanoparticles that manage to penetrate the lungs of breathing mice are only able to penetrate the epithelial–endothelial barrier

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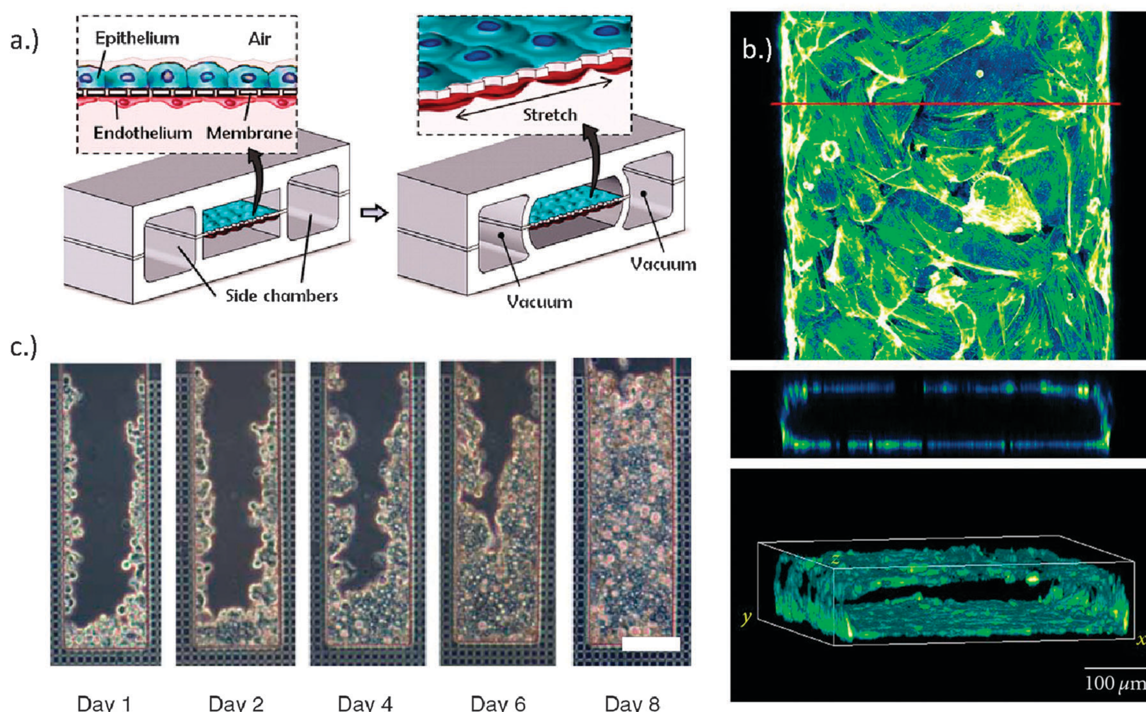

Fig. 2 Several examples of organs-on-chips. (a) Schematic overview of a lung-on-a-chip. Epithelial cells and endothelial cells are co-cultured on a permeable membrane. The membrane can be subjected to mechanical stretch by applying a vacuum in the side chambers. Left: device in a resting state. Right: device with vacuum applied. Reproduced with permission from Huh *et al.*[21] (b) Actin staining of endothelial cells in a microfluidic channel. By culturing cells in a microfluidic channel, they can be routinely subjected to fluid flow. Top, pseudo-colored confocal fluorescence microscopy top view of the channel. Middle, reconstituted cross-section of the channel at the location of the red line in the top image. Bottom, three-dimensional reconstruction of the confocal image in the top. Reproduced from Van der Meer *et al.*[37] (c) Culture of human hepatoma cells in a constricted, microfluidic compartment. The grids on the three sides of the compartment are the perfusion barriers that separate the culture compartment from the medium channel. Scale bar, 100 μm. Reproduced with permission from Zhang *et al.*[49]

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of the microfluidic culture model when mechanical stress is applied, not when the culture is kept under static conditions.[21] This result is a clear indication that organs-on-chips can reveal physiological phenomena that would normally remain undetected.

To sum up, microengineered models of the lung feature both the cellular organization and several well-defined important mechanical factors that play a role in lung physiology. Meanwhile, the models are still simple and small.

Blood vessel-on-a-chip

Vascular endothelium forms the inner lining of all blood vessels, from large-diameter arteries to capillaries. Fluid flow and the resultant wall shear stress are very important factors in the physiology of vascular endothelial cells. Moreover, shear stress is known to be involved in drug-induced vascular injury,[33] the pathogenesis of atherosclerosis[34] and the formation of thromboemboli.[35]

There is already a large body of literature on the endothelial response to shear stress.[36] Most studies employ a macroscopic experimental set-up that consists of a parallel plate flow chamber connected to a peristaltic pump or a syringe pump. After culturing endothelial cells in the chamber and subjecting them to different regimes of shear stress, the functional response and the underlying mechanisms are analyzed. In other words, the studies that include shear stress in their experimental design focus almost exclusively on the direct effects of shear stress on endothelial biology. The shear stress stimulus is not routinely included when studying other—seemingly unrelated—aspects of endothelial biology, simply because the parallel plate flow chambers are relatively cumbersome and low-throughput set-ups.

Because microfluidic technology enables the manipulation of fluids on the microscale, it should be no surprise that many studies have appeared in which microfluidic devices are employed to study vascular biology and, more specifically, the endothelial response to shear stress.[37] Similar to the work in macroscopic flow chambers, the first reports of microfluidic versions of parallel plate flow chambers also focused solely on the immediate effects of shear stress on endothelial cell physiology and morphology.[22,38,39] These early studies already highlighted the fact that microfluidic devices allow for parallelization and automation, while using minimal amounts of reagents.

Because the inclusion of shear stress as a culture condition proved to be relatively simple in microfluidic devices, studies were then performed in which the shear stress stimulus was included routinely, but that focused on other aspects of endothelial biology like the uptake of cholesterol or the cytokine-induced capture of monocytes.[40,41] These are typical illustrations of how microengineering can increase the realism of an *in vitro* model, without affecting the implicit robustness of such a model. The increased realism and potential for

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parallelization of vascular models may contribute greatly to both fundamental research on the development of vascular injury and disease, as well as to the development of safe drugs that do not trigger endothelial injury or inflammation.

In summary, microengineering allows researchers to routinely mimic the flow of blood in their studies of vascular endothelium. Inclusion of this culture parameter leads to more realistic *in vitro* models of blood vessels.

Liver-on-a-chip

From a toxicological and a pharmaceutical perspective, the liver is one of the most interesting organs to study. Hepatoxicity is a major problem in clinical drug development and is one of the major reasons for withdrawing a drug from the market after introduction.[42] More reliable toxicity screening early in the drug development process would therefore have a big economic impact.

There have been numerous reports of microfabricated models of liver tissue that illustrate the potential for high-throughput toxicity screening, as well as for investigating hepatic physiology.[43,44] The main advantage of microfabricated culture systems for hepatic cells is that it allows the formation of hepatic clusters of a few hundred micrometres in size. Primary hepatic cells need the interaction that is provided by such clusters to act as a functional unit and retain their phenotype for longer periods of time. An elegant example of this phenomenon is given by a study in which hepatic cells were grown on micropatterned spots, surrounded by fibroblasts. Micropatterned hepatocytes maintain their liver-specific functions, whereas a lack of patterning leads to a rapid loss of hepatic characteristics.[45] Similar studies have been performed with hepatocytes grown in clusters or with cells patterned by dielectrophoresis.[46–49] This is a typical illustration of the self-organizing capacity of tissue. Just by altering a few aspects of the culture microenvironment, the increase in biological realism can be dramatic. Still, there is plenty of room for improving the geometry and organization of livers-on-chips even further by incorporating bile ducts and well-defined oxygen gradients.

In short, the impact of microengineering on *in vitro* liver models is mainly focused on the geometry and organization of the tissue. Moreover, because microfabricated liver models have great potential in pharmaceutical screening, researchers in this research area are well aware of the need for high-throughput in the use of their models. There is a clear focus on compatibility with pipetting robots[45] and on parallelization.[47]

Other tissues

The list of examples discussed here is by no means exhaustive. Initial attempts have been undertaken to develop microengineered models of other tissues.

In microengineered models of the kidney, cultured renal epithelial cells are exposed to physiological levels of fluid flow and to a two-compartment microenvironment.[50,51] As a result, the cultured cells form well-differentiated, functional monolayers with clear apical-basolateral polarity.

The engineering of microenvironments for heart tissue has focused on the nanotopography of the tissue culture surface, mechanical stimulation by means of fluid shear stress, stretch and contraction, as well as electrical stimulation by integrated electrodes.[52] All these engineered parameters were shown to contribute to hallmarks of cardiac differentiation, such as cell alignment, marker protein expression and contraction.

Recently, a microengineered construct that mimics the geometry of intestinal villi was reported: a study that paves the way for more realistic *in vitro* models of the intestine.[53]

Various microfluidic systems have been reported for culturing pancreatic islets of Langerhans in a confined geometry. Because these cell clusters have an important endocrine function, the microfluidic systems generally include measurement techniques to analyze the kinetics of hormone secretion.[54]

Finally, microengineered devices have also been shown to be relevant in constructing neuronal models.[55] Especially the directed growth of axons in two or three dimensions by generating surface-bound or soluble biochemical gradients has proved to be useful. Moreover, such directed growth can be combined with electrical stimulation by embedded electrodes.

In short, microengineering is being employed to increase the physiological realism of *in vitro* models of various tissues. All of these novel models include important organ-level features, such as tissue geometry, mechanical and electrical stimulation or biochemical gradients.

The future of organs-on-chips

The first generation of organs-on-chips have demonstrated that it is possible to unite simplicity and realism in one *in vitro* tissue model by applying microtechnology. This means that the devices have great potential to change the landscape of *in vitro* testing for fundamental biology, drug development and toxicology. So how to accomplish such a change? Where should the field move from here? Basically, three factors should be considered: what design philosophy to follow, how to merge technology with biology and how to connect to the end-users.

The future: design philosophy

The first successes of microfabricated tissue models have led researchers to philosophize about coupling and combining these organs-on-chips within a single device. This would yield a device with complex, interacting organ systems and in the end maybe even a 'body-on-a-chip'.[56–62] The idea seems alluring: it would yield a truly comprehensive *in vitro* system with the potential to replace certain animal tests. Because of the small size of bodies-on-chips, they can potentially be parallelized and used in early stages of drug development, in which the pharmacokinetics and pharmacodynamics of dozens of substances need to be tested.[58] Moreover, bodies-on-chips can potentially be integrated in portable analysis platforms, or 'labs-on-chips',[63] which can then be used to screen for toxicity of substances and environmental factors in the field.

Even though the extensive coupling of tissue models within one device is a very interesting direction of research in its own right, it is a pitfall that should be side-stepped when trying to develop a robust tissue model. From a biological perspective, adding more and more tissues to a microfabricated model is the equivalent of complicating the culture conditions of the cells of interest. Such an increased variation of the initial

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conditions will blur the results of measurements and the underlying mechanisms that yield those results. Or, in reference to the first part of this review, we would arrive at the simplicity-*versus*-realism impasse again.

So how to proceed while side-stepping this pitfall of adding biological complexity? The design philosophy that has been used for the first organs-on-chips points us in the right direction. If we want to mimic blood flow, we include a pump, not a micro-cultured heart;[22] if we want to mimic respiration, we include a mechanical stretcher, not a culture of muscle cells;[21] if we want to mimic the generation of drug metabolites, we include an electrochemical cell, not a culture of hepatic cells,[64] *etc*. As long as the complexity is of a non-biological nature, it can be controlled, steered, quantified and employed by the researcher while doing the actual measurements solely on the biological tissue in the device. In other words, only the immediate objects of experimentation should be included in the form of biological tissue; the other, physiologically relevant, parameters should be engineered and under control of the researcher. In this way, the model becomes more realistic without blurring the results of measurements (Fig. 3).

Of course, when applying this approach, one must always be aware that the 'realistic' model—although more physiologically relevant than conventional cell culture models—is still a simplification of the actual microenvironment of the tissue of interest. Tissue physiology will never be comprehensively mimicked by microenvironment engineering. For example, the role of smooth muscle tissue goes far beyond that of a simple mechanical stretcher. The tissue is actively involved in shaping the microenvironment of the surrounding cells by proliferation and secretion of growth factors.[65] In turn, its contractile function is strongly affected by factors from the surrounding tissue.[66] Similar points can be made about other tissues, such as cardiac tissue and hepatic tissue. Given this risk of ignoring or misrepresenting essential physiological parameters of the microenvironment, it is very important to validate organs-on-chips thoroughly by comparing the tissue models with the *in vivo* tissues of interest.

Moreover, in many cases, physiological parameters can be essential for cell function, but too complex to mimic by engineering. For example, the nature of some close interactions between two cell types, like immune cells with other tissues, has not been unraveled to a level where one of the cell types can simply be represented by a set of engineered parameters. In such cases, both cell types will still have to be included in the model, despite the resultant decrease in robustness.

To sum up, physiological parameters of the microenvironment should be incorporated into organs-on-chips by artificial engineering as much as possible, but caution should be exercised not to oversimplify the model. This should be done by validating the effects of the engineered physiological aspects, as well as by including more cell types when their full spectrum of physiological parameters is needed for the model to generate meaningful results.

Apart from these considerations about how to include physiological parameters, there are a number of other issues with the current generation of organs-on-chips that need to be resolved. First of all, because organs-on-chips are so small, dynamic culturing is almost always a requirement. This means that the set-ups as a whole are still relatively bulky, with tubing, connections, syringes and pumps hooked up to them. There have been a number of reports of integrated pumps and medium reservoirs in microfabricated devices,[47,67,68] as well as various passive pumping methods based on capillary force,[69] surface tension[70,71] and gravity,[72] but none of these have become a widespread standard, yet. The field is in dire need of simple, integratable, particle-tolerant micropump technology. Additionally, virtually all organs-on-chips are currently produced in polydimethylsiloxane. Even though this is a useful material for the rapid preparation of prototypes, it is porous, hydrophobic and absorbs many small molecules and drugs. The next generation of microfabricated tissue models should therefore be constructed using glass or more inert polymers, like polystyrene or cyclic olefin copolymer.[73,74] Thirdly, a major challenge in the field of organs-on-chips is the development of downstream and online analysis of biological parameters. Because of the small dimensions and small sample sizes, many current biochemical techniques are not compatible with organs-on-chips. Therefore, the development of new organs-on-chips should go hand-in-hand with the development of new microfluidic technology for biochemical and functional analyses. Many micro-scale versions of well-known biological assays have already appeared in the literature from flow cytometry[75] to polymerase chain reaction,[76] Western blotting[77] and DNA electrophoresis.[78] However, most of these techniques have not matured into a stage where they are standardized and able to compete with conventional, bench-top biochemical tools. More research in the direction of integrated analytical technology can increase the power and versatility of organs-on-chips as tissue models.

Finally, research groups that develop organs-on-chips and downstream methods for biochemical analysis currently all employ their own devices and platforms. This has resulted in a relatively fragmented field with technology that is hard to adopt, adapt and integrate into a unified platform. In the future, it would be very advantageous to develop a standardized platform, into which pumps, culture chambers and bioanalytical units can be plugged. With such a platform, new functionality can be rapidly developed by combining parts of the existing, collective toolbox. By following such a modular approach, most efforts of the research community can be combined easily, decreasing development times and facilitating use and application of the technology.

In short, the next generation of organs-on-chips should remain faithful to the original design philosophy of controlled, artificial engineering of the tissue microenvironment. Moreover, the devices should become more standardized in terms of design, pumps, materials and downstream analysis. Such standardization must eventually lead to a modular development platform.

The future: merging technology and biology

Organs-on-chips rely on an intricate interplay between engineering and biology. From the examples in this review, it is clear that the engineering of very basic aspects of the culture microenvironment can induce a realistic tissue phenotype. Currently, the engineering of the culture microenvironment is a very static, one-sided process. This means that the culture parameters are set at fixed values that do not change over the course of the experiment and that are not dependent on any feedback from the cultured cells. However, in

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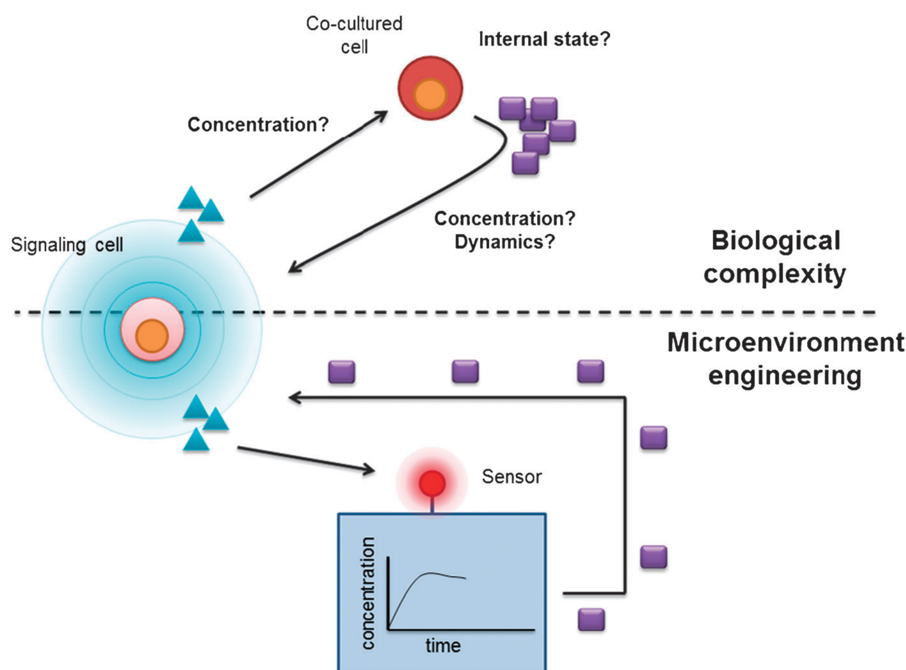

Fig. 3 Physiologically realistic models can be achieved either by adding biological complexity or by engineering the microenvironment. Adding biological complexity introduces several unpredictable factors in the model, whereas microenvironment engineering allows for continuous quantification and control over many culture parameters. Therefore, microenvironment engineering generates biological realism without adding complexity to the model.

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many processes, such as the differentiation of stem cells, one fixed set of parameters is not enough and the culture parameters need to be altered over time to guide the cells towards the required differentiated state.[79,80] Moreover, the cells themselves can give valuable information about their response to the imposed microenvironment. For example, smooth muscle cells release inflammatory factors when they are subjected to aberrant mechanical stresses and endothelial cells release nitric oxide when subjected to high shear stress.[81,82] By 'listening' to the cells, the culture parameters can be adapted over time, thereby making the interplay between engineering and biology a two-way process and increasing the realism and stability of the model.

Microfluidic engineering harbors enormous potential to generate automated, spatiotemporally dynamic culture conditions. A lot of examples have already appeared in the literature that describe microfluidic devices that are capable of local delivery of soluble factors.[83–85] Moreover, because the microfluidic devices rely on pumping for refreshing their contents, this local delivery can also be varied over time by programming dynamic pumping regimes.

The level of engineering that is used in the current generation of organs-on-chips is very useful, but almost rudimentary in terms of spatiotemporal dynamics. Next generations of the devices will be a lot more dynamic, incorporating sensors and response elements, all connected by software that is designed on the principles of control theory. In this way, the models will become small technological–biological hybrid systems, entering the realm of cybernetic-organics.[86] The first examples of such microtechnology-driven homeostatic devices have already appeared in the literature.[87,88]

Interestingly, the engineering of a spatiotemporal realistic microenvironment in the model could even lead to a decrease in the spread of measurement results compared to the current simple *in vitro* models. The incorporation of sensors, pumps and homeostatic feedback loops can maintain levels of medium flow, pH, oxygen, glucose and growth factors constant over the course of the experiment, thereby lowering the variation under culturing conditions.

Moreover, engineering a responsive microenvironment may lead to increased insights in human physiology. In order to design biologically responsive models, a more profound understanding is needed of the spatiotemporal dynamics and the nature of signaling loops and networks in biological systems. Organs-on-chips are interesting research platforms to study such physiological feedback mechanisms.

To sum up, future organ-on-a-chip devices should become more spatiotemporally dynamic and responsive when engineering a tissue microenvironment. Such a dynamic response can be engineered by implementing signaling networks in the form of sensors, algorithmic control loops and response elements, such as pumps, heating elements and electrochemical cells.

The future: end-users

The end-users of microfabricated tissue models will be biologists, toxicologists and the pharmaceutical industry. Biologists are mainly interested in the structure and function of the human body. These scientists invest a lot of time in isolating and culturing human tissue, generating and differentiating stem cells, modifying the cells by DNA transfection or by RNAi, and developing assays to perform functional tests on the resulting material. So in order to become accepted by the biological research community, microfabricated *in vitro* models should be compatible with as many conventional techniques as possible,

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should not complicate existing laboratory protocols and should offer immediate added value compared to the current laboratory tools. When the first generation of microfabricated tissue models is ready to be introduced in cell biological labs, their formats should be compatible with conventional spectrophotometers and high magnification microscopy.[89] Moreover, the cellular content of the devices should be accessible for detachment or lysis, so that detailed biochemical analysis can be performed by polymerase chain reaction, immunoassays and microarrays. Additionally, organs-on-chips have not been developed and streamlined up to a point where they can be used in the standard biochemical laboratory. Usually, the devices require specific expertise and equipment to be seeded, operated and analyzed. For example, seeding cells in a microfluidic device requires gentle pipetting or pumping, high cell concentrations, careful removal of air bubbles, air bubble-free connections for tubing, constant refreshment of medium, degassing of the inflowing medium, setting up pumps inside or near an incubator, *etc*. In short, even though organs-on-chips offer a simple, well-defined microenvironment for the cells of interest, the laboratory protocols to produce this microenvironment are definitely not straightforward. In order to solve this, there are two solutions that can be implemented concurrently. The devices can be made more simple to operate, drawing inspiration from current microtiter plates and other conventional culture methods. Alternatively, the delicate and complicated operating protocols can remain in place, but the procedures must be shielded from the end-users by a well-designed user interface. As mentioned earlier, the development of a standardized platform that integrates many functions could be of great help here.

The other end-users of organ-on-a-chip-technology—toxicologists and the pharmaceutical industry—have a strong need for realistic models that deliver information on pharmacokinetics and pharmacodynamics with a very high throughput. For these end-users, the promise of parallelization and automated analysis that is found in many papers on organs-on-chips will need to be materialized. The best way to accomplish this is to make sure that future organs-on-chips are compatible with conventional automated technology, such as pipetting robots.[90] This means that parallelized organs-on-chips should preferably be developed in a conventional microtiter plate format and be (semi-)open-ended to allow robots to access the contents of the devices.[91] Moreover, in order to analyze the pharmacokinetic behavior of their candidate drugs, the pharmaceutical industry requires downstream compatibility of microfluidic tissue models with mass spectrometry. Luckily, miniaturization of mass spectrometry interfacing is a rapidly evolving field of research.[92,93]

It is needless to say that the success of organs-on-chips will depend heavily on the acceptance of these models by the various end-users. Biologists will only embrace new tools that have proven their weight by facilitating breakthroughs in biological research. Even though organs-on-chips have huge potential to revolutionize *in vitro* research by increasing control over realistic culture conditions, by decreasing the size of current assays and by parallelization for increased throughput, acceptance by the end-users will not take place automatically. Active efforts are needed to reach out to the biological research community and the pharmaceutical industry, to engage with them in dialogue about the further development of the devices, to set up collaborations and to understand which important physiological and pharmaceutical research areas can easily be explored with organs-on-chips.

The future: summarized

To sum up, further development of organs-on-chips will need to rely on the unique concept of microenvironment engineering. This means that future devices will include more advanced combinations of microelectrical, micromechanical and microfluidic components. Moreover, the physiologically relevant microenvironment that these engineered components produce will be more spatiotemporally dynamic and will rely on concepts from control theory. Finally, the devices will become more standardized, modular, automated and parallelized. Such a trend towards 'low maintenance-high throughput' is necessary for the devices to have an impact on biological fields like human physiology, cell biology, toxicology and drug development. The devices should be easy to operate for the biological end-users and need to be compatible with current biological laboratory technology.

Conclusion

The new microfabricated *in vitro* tissue models, or organs-on-chips, are promising tools for studying physiology and pathogenesis in simple, yet realistic systems. The first results show clear improvements compared to results of experiments with conventional *in vitro* models. For future development, the focus should lie on increasing compatibility with current biochemical techniques, integrating analytical technology and standardizing the design principles. If developed in these directions, organs-on-chips definitely have the potential to soon become well-accepted by biologists worldwide and become key elements in the drug development process of the pharmaceutical industry. Meanwhile, the first generation of organs-on-chips offers only a glimpse of the full potential of this novel class of *in vitro* models. It will be interesting to see what level of integration of biology and technology can be achieved with even more sophisticated engineering in the future.

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We apologize to the many authors in the field whose excellent work was not cited in this review. The main motivation to write this review was to offer our perspective on the flourishing field of microengineered tissue models and organs-on-chips. Our selection of references was primarily based on how helpful the articles were to get our points across, not just on the merit of the work described in them.

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