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SPOTLIGHT

Developmentally inspired human 'organs on chips'

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ABSTRACT

Although initially developed to replace animal testing in drug development, human 'organ on a chip' (organ chip) microfluidic culture technology offers a new tool for studying tissue development and pathophysiology, which has brought us one step closer to carrying out human experimentation *in vitro*. In this Spotlight article, I discuss the central role that developmental biology played in the early stages of organ-chip technology, and how these models have led to new insights into human physiology and disease mechanisms. Advantages and disadvantages of the organ-chip approach relative to organoids and other human cell cultures are also discussed.

KEY WORDS: 'Organ on a chip', Microfluidic, Organoid, Mechanical, Multiphysiological system, Mechanobiology

Introduction

Human 'organs on chips' (organ chips) are microfluidic cell culture devices with separate parenchymal and vascular compartments lined by living human cells that mimic the multicellular architecture, tissue-tissue interfaces and relevant physical microenvironment of key functional units of living organs, while providing dynamic vascular perfusion *in vitro*. They are called 'chips' because they were originally fabricated using methods adapted from those used for manufacturing of computer microchips. This technology is exciting because it provides a dynamic window into molecular scale activities inside living cells within a relevant human tissue and organ context, while permitting separate access to the parenchymal and vascular compartments. Equally important, because all culture control parameters can be varied individually, it offers a new tool for studying how cellular, molecular, chemical and physical cues work alone, and in combination, to influence human tissue development and disease.

Microfluidic devices have been used for cell culture for almost 20 years; however, prior to the development of multi-compartment organ chips, cells were simply cultured on conventional, rigid, substrates placed within a single flow channel. Over time, these single-channel microfluidic culture devices, and more complex ones that incorporate different types of cells with or without extracellular matrix (ECM) gels, have been developed as 'multiphysiological systems' for modeling tissue pathophysiology; these systems are also sometimes referred to organ chips (reviewed by Bhatia and Ingber, 2014). These simpler microfluidic devices have been used to effectively model tissue-level functions as well as important related developmental processes, such as tumor cell invasion, immune cell migration and angiogenesis (reviewed by Bhatia and Ingber, 2014).

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However, the first microfluidic organ chip device to accomplish the goal of recapitulating organ-level functionality by creating separate parenchymal and vascular compartments, tissue-tissue interfaces and a physiologically relevant organ microenvironment, while maintaining vascular perfusion, was a human breathing lung alveolus chip (Fig. 1A,B; Huh et al., 2010). Since this study was published, multi-channel design approaches have been used by multiple research groups to build microfluidic organ-chip models of the human lung small airway, skin, kidney, intestine, placenta, blood-retinal barrier, blood-brain barrier, neurovascular unit and neuromuscular unit, among others (Kim et al., 2012; Achyuta et al., 2013; Abaci et al., 2015; Benam et al., 2016a; Lee et al., 2016; Musah et al., 2017; Yeste et al., 2017; Wang et al., 2017; Workman et al., 2018; Kasendra et al., 2018; Sances et al., 2018; reviewed by Bhatia and Ingber, 2014).

Role of developmental biology in the origin of organ chips

The story of how organ chips developed is commonly conveyed as one involving the intersection between microsystems engineering and cell biology. Although this is true, the path to organ chips was equally influenced by developmental biology and, in particular, the key role that mechanical forces play in controlling cell fate and tissue morphogenesis. In fact, it was the challenge of convincing other biologists that cell shape distortion is a physiologically relevant control element that led me to collaborate with George Whitesides, and to first apply his soft lithography-based microchip manufacturing approach to engineer culture substrates that can control cell shape and function (Singhvi et al., 1994). Shu Takayama worked with us and used soft lithography to create microfluidic channels and to culture cells under flow inside these channels (Takayama et al., 2001). Later, when Shu formed his own laboratory, he and his student Dan Huh created a microfluidic device with an air-filled channel of similar size and shape to a small lung bronchiole. When they flowed droplets of fluid through it to mimic mucus plugs, the device generated a detectable noise, which precisely matched the 'crackle' sound that I was taught to listen for in patients with pneumonia when I was a medical student. When Dan Huh applied to be a postdoctoral fellow in my laboratory, I told him how impressed I was with this breakthrough. However, I asked, 'why don't we build a real living, breathing 'lung on a chip' lined by human lung cells and vascular endothelium interfaced in the center of two microfluidic channels?'. And this is precisely what Dan did, which led to our lung alveolus chip publication (Huh et al., 2010).

Our approach to building this organ chip was to distill down the problem to its simplest developmental principles: what makes an organ an organ? The answer for us was that it involves physical interfacing and synergistic functional interplay between two or more tissues, and a vascular endothelium is almost always one of these tissues. Furthermore, because of our deep belief that mechanical forces govern cell and tissue development, it was crucial to replicate organ-relevant physical cues, which in the case of the lung involved surface tension at an air-liquid interface, as well as both fluid flow through the vascular lumen and cyclic mechanical distortion of the

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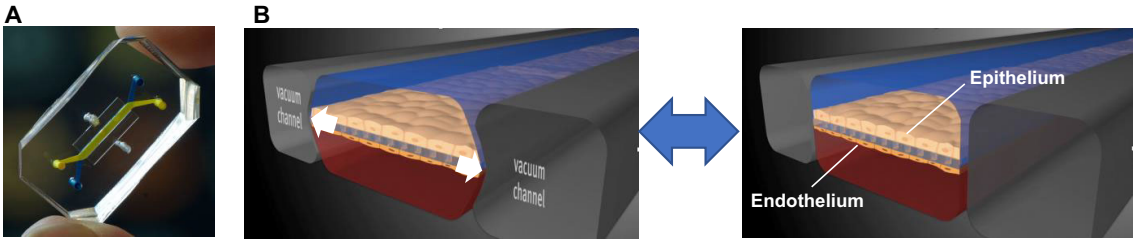

Fig. 1. A multi-channel human organ-chip model of the lung alveolus. (A) The lung chip is made of an optically clear, flexible, silicone rubber polymer the size of a computer memory stick with two tiny hollow channels running parallel along its length separated by a flexible porous membrane of the same material (channels are filled with blue and yellow dyes). (B) Diagrammatic cross-sections of the lung chip showing how living human alveolar epithelium is cultured on top of the porous membrane, which is coated with ECM, and air (blue) is introduced into the overlying space to create an air-liquid interface, while human microvascular endothelium is grown on the opposite side of the same membrane under continuous medium flow (red). These two channels are also surrounded on both sides by hollow vacuum chambers through which cyclic suction is applied (left image) and released (right image) to mimic breathing motions by rhythmically extending and relaxing the flexible porous membrane and attached lung tissues. In this manner, this device essentially creates a living, breathing, 3D cross-section of a major functional unit of a living human organ – the lung alveolus. A similar approach can be applied to model major functional units of other human organs.

tissue-tissue interface due to breathing motions. When we later created a model of the human intestine, we similarly applied cyclic mechanical strain, but at different rates and degrees, to mimic effects of peristalsis-like motions (Kim et al., 2012; Kasendra et al., 2018); when we built a kidney glomerulus chip (Musah et al., 2017), we recreated the deformations this organ unit experiences due to pulsatile blood flow with every beat of the heart. Most importantly, in all of these studies, these mechanical perturbations were absolutely required to drive cell and tissue differentiation, and to robustly mimic human organ-level physiology, as well as pathophysiology.

Developmental biology has also contributed significantly to the success of organ chips in other ways. For example, one of the key challenges in this field is to obtain highly functional, human, organ-specific parenchymal cells. Many adult cell types are available commercially; however, tissue-specific organoids also can be created by isolating stem cells from patient biopsies, and this approach has been leveraged as a cell source to create functional human intestine chips that form highly differentiated villus structures (Kasendra et al., 2018). Induced pluripotent stem cell (iPSC) approaches have also been leveraged to create various types of specialized cells for organ chip studies, including cardiomyocytes, kidney podocytes, brain microvascular endothelial cells, and intestinal enterocytes (Wang et al., 2014, 2017; Musah et al., 2017; Workman et al., 2018), but their clinical relevance is sometimes questioned because most iPSCs generated *in vitro* remain fetal-like or neonatal in nature. Importantly, culturing iPSC-derived motoneurons and brain microvascular endothelial cells together in an organ chip model of the neuromuscular unit significantly enhanced function and *in vivo*-like maturation of spinal cord neural tissue (Sances et al., 2018). When cultured on chip, human iPSC-derived kidney podocytes also matured further by extending long foot processes much as they do *in vivo* (Musah et al., 2017). Hence, organ chip approaches could be used to enhance stem cell differentiation *in vitro* and to study patient-specific developmental responses for personalized medical applications in the future.

Another key principle behind organ chip engineering is harnessing the fundamental processes of cellular self-assembly, sorting, cellular differentiation and tissue morphogenesis that are central to embryological development. When different cell types are placed in the correct microenvironment with appropriate positioning of tissues relative to one another, they spontaneously accumulate ECM along their interface, switch on developmental programs and self-organize, resulting in formation of highly polarized and differentiated epithelial tissue structures that closely resemble those seen in the organs of our bodies, such as mucus and surfactant-producing alveolar epithelium (Huh et al., 2010), ciliated pseudostratified airway epithelium (Benam et al., 2016a), 3D finger-like intestinal villi (Kim et al., 2012; Kasendra et al., 2018; Workman et al., 2018) and reconstitution of podocyte-ECM-endothelial contacts of the kidney glomerulus (Musah et al., 2017).

A tool for dissecting human development and disease

Organ chips are also useful for analyzing the biological and biophysical mechanisms that underlie organ-level physiology and disease development. For example, in the intestine chip, fluid flow was found to be crucial for villus morphogenesis and mucus production (Kim et al., 2012; Kasendra et al., 2018), whereas cyclic peristalsis-like deformations were responsible for suppressing overgrowth of commensal microbes and breakdown of the intestinal barrier (Kim et al., 2016). In the lung alveolus chip, absorption of nanoparticulates and associated inflammation were highly sensitive to breathing motions, as was the development of pulmonary edema (Huh et al., 2010; Huh et al., 2012). Physiological breathing motions were also unexpectedly found to inhibit the growth and invasion of lung cancer cells growing orthotopically in this chip (Hassell et al., 2017). When the vascular channels of these organ chips are lined by living endothelium, it is even possible to flow human whole blood through these devices without requiring anticoagulants. Using this setup, induction of pulmonary thrombosis by lipopolysaccharide endotoxin was shown to not be a direct effect of the endothelium or blood; instead it resulted from induction of cytokine production by the epithelium, which then activated the endothelium to make it pro-thrombotic as a result of this tissue-tissue crosstalk (Jain et al., 2018).

But the most powerful feature of organ chips is that they essentially enable a synthetic biology approach to investigate mechanisms at the cell, tissue and organ levels. A single tissue can be cultured ‘on chip’: if it fully mimics *in vivo*-like functionality, then all of the other cell types (which had been previously assumed to be crucial) are not required for this behavior. One example is the finding that pulmonary vascular leakage induced as a side effect of the cancer drug interleukin 2 can be induced in the lung chip even though immune cells are not present (Huh et al., 2012), thus demonstrating that the previously held assumption of their crucial role in this process is incorrect. Conversely, although endotoxin induces intestinal injury and loss of barrier function *in vivo*, it does not produce this effect in a chip lined only by intestinal epithelial cells; in this case, immune cells also must be present (Kim et al., 2016). Furthermore, because there is continuous fluid flow through

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the lumen of the vascular channel (and sometimes in the parenchymal tissue channel as well), it is possible to continually analyze the effluent for secreted factors, such as cytokines, that mediate physiological, pathophysiological or developmental responses, and their concentrations can be measured. In the intestine chip, this approach revealed a combination of four distinct cytokines (IL6, IL8, IL1, and TNFα) whose levels increased under conditions that led to villus blunting and disruption of barrier function (Kim et al., 2016). Importantly, this response could be replicated when these four cytokines were added back at the same concentrations, but not when they were administered individually. Gradients of chemicals and gases (e.g. oxygen) also can be easily generated with these two-channel chip devices by altering their concentrations in the medium perfused through the parallel channels.

Thus, organ chips provide a tool that may be useful to analyze the contributions of key chemical, molecular, cellular and physical factors to both tissue development and disease, as well as analyze how they work both alone and in combination. In addition, because organ chips support functional differentiation, they also may be used to dissect fundamental issues relating to the control of key developmental processes such as the regulation of cell lineage choice, differentiation, cell positioning, compartmentalization, planar cell polarity, morphogenesis and many more in forming human tissues. By fluidically linking multiple, dual-channel, organ chips via their endothelium-lined vascular channel, it might be possible to analyze how physiological signaling between different organ systems contributes to developmental processes in the future as well.

Advantages and disadvantages relative to other human cell culture models

From the discussion above, it is clear that multi-channel organ chips offer many advantages over conventional human culture models because they provide fluid flow, relevant mechanical cues, an organ-level tissue-tissue interface, and separate access to parenchymal and vascular compartments; and they can be studied using virtually any analytical technique that is used in conventional cultures, including high-resolution real-time fluorescence imaging, multi-omics, mass spectroscopy and many others. The chip approach also offers many advantages relative to the popular organoid culture method because it provides continuous access to the apical lumen of both the epithelial and vascular channels, reconstitutes tissue-tissue interfaces, recreates the dynamic physical microenvironment, supports long-term co-culture of commensal microbes and enables physiologically relevant recruitment of circulating immune cells under flow (Kim et al., 2012, 2016; Huh et al., 2010; Benam et al., 2016a), which is difficult or impossible to accomplish using organoids. Organ chips also permit investigators to use imaging to return to the same location at the same tissue-tissue interface every day, whereas the shape of organoids changes constantly over time. In addition, multi-electrode arrays analyzing cell electrical potentials, and electrodes for measuring transepithelial electrical resistance (TEER), can be integrated in organ chips (Maoz et al., 2017), whereas this is not possible with organoid cultures or more-complex 3D multiphysiological systems.

However, organ chips also have their limitations. For example, it is much easier to multiplex organoids, larger multiphysiological systems (e.g. that incorporate Transwell inserts) or simpler single-channel microfluidic devices to carry out higher-throughput studies, produce results more quickly and increase the numbers of experimental replicates. Simpler devices that contain only one cell type grown in 3D ECM gels also are more useful for studying dynamic morphogenetic processes, such as blood vessel formation, as well as migration of immune cells and cancer cells through the interstitium (reviewed by Bhatia and Ingber, 2014). However, stromal cells and ECM gels can be integrated into multi-channel organ-chip interfaces. For example, fibroblasts have been integrated in a model of early stage human breast cancer (Choi et al., 2015). Most importantly, these more-complex organ chips with tissue-tissue interfaces offer a much higher level of physiological mimicry for human organ function than most other multiphysiological systems, as they have been repeatedly shown to replicate results from healthy and diseased animal models (Huh et al., 2012; Benam et al., 2016a; Jain et al., 2018), and even results from human studies (Benam et al., 2016b; Barrile et al., 2018). They should not be viewed as replacements for other culture models; instead, they should be viewed as alternatives to animal models and integrated into laboratories as a form of preclinical testing that more closely approximates human experimentation *in vitro*. Finally, it is important to note that organ chips can exhibit significant variation and inconsistency between different manufacturing batches, different laboratories and even different fabricators in the same group. However, organ chips are now being commercialized by multiple companies, which are able to produce more robust and consistent devices; this should lead to greatly increased accessibility to laboratories and industry around the world.

Concluding remarks

Human organ chips that recreate organ-level physical microenvironments, tissue-tissue interfaces and vascular perfusion offer a powerful new approach to study human biology. Insights from developmental biology played a central role in their creation, but these unique culture models may now in turn help facilitate developmental studies in a more human-relevant context. The more we learn about developmental control and stem cell biology, the more we can increase the sophistication and functionality of these biomimetic microfluidic culture devices. One of the unique properties of the dual channel organ chips is that they enable stable co-culture of living human cells with living commensal microbes for extended times (days to weeks), whereas organoids or conventional cultures rapidly die due to contamination. Thus, this is currently the only method that can allow us to explore how complex human microbiota influence developmental control in human tissues over time. It is also possible to build organ chips with cells from different species, e.g. mouse versus human, to experimentally determine the extent to which mechanisms of tissue development and functional regulation are conserved. Where they are not, we may now have a tool that can be used to study and define these mechanisms in a human-specific setting.

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Competing interests

D.E.I. holds equity in Emulate and chairs its scientific advisory board.

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