Cell

Reverse Engineering Human Pathophysiology with Organs-on-Chips

Donald E. Ingber[1,2,3,*]
[1]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115, USA
[2]Vascular Biology Program, Departments of Pathology & Surgery, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA
[3]Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, MA 02138, USA
*Correspondence: don.ingber@wyss.harvard.edu
http://dx.doi.org/10.1016/j.cell.2016.02.049

While studies of cultured cells have led to new insights into biological control, greater understanding of human pathophysiology requires the development of experimental systems that permit analysis of intercellular communications and tissue-tissue interactions in a more relevant organ context. Human organs-on-chips offer a potentially powerful new approach to confront this long-standing problem.

Biomedical research seeks to understand human physiology and how cellular behaviors become deregulated during disease development to discover more effective diagnostics, therapeutics, and means of disease prevention. To gain insight into the inner workings of life, biologists have reduced complex human organ systems into smaller and simpler subsystems such that most in vitro studies now involve analysis of only one type of cell or tissue cultured in isolation. While this approach has led to useful insights into cellular regulation, physiological control involves higher order intercellular communication, involving chemical, molecular, physical, and electrical information transfer between cells in different tissues, as well as functional coupling between different organs, and with each systems jump, new functions emerge. In addition, we now know that the normal microbiome that lives on and in our bodies is critical for health and disease. Thus, to gain a deeper understanding of human physiology and pathophysiology, we need to develop new in vitro methods to study cellular control in a more relevant, organ and multi-organ context.

Animal models are often used to address this challenge, but they often fail to faithfully mimic human response (Leist and Hartung, 2013). Three dimensional (3D) "organoid" cultures have been developed that support formation of higher order tissue-like (Sato and Clevers, 2013) and even organ-like (Lancaster et al., 2013) structures; however, organoids lack many features that are critical for organ function, such as vascular perfusion, mechanical cues (e.g., breathing motions in lung), circulating immune cells, and the ability to co-culture normal microbiome for extended times. We currently do not have the technology to build a fully functional, living replica of a whole organ with the correct 3D spatial relationships and a one-to-one correspondence of part to whole down to the cellular and molecular levels. But even if we could accomplish this, it would not necessarily provide a way to selectively remove or add back individual cell and molecular components to analyze their contribution to organ-level behaviors, to visualize molecular scale activities at high resolution in real-time, or to carry out biochemical and genetic analysis of the interacting tissues individually, which are all are crucial for gaining insight into how intercellular signal transfer and tissue-tissue interactions contribute to physiological control. But there is another way to do this; we can "reverse engineer" living organs.

Reverse Engineering

Reverse engineering is the process of extracting basic design information, usually from a man-made system or device, and using this knowledge to reproduce a new equivalent or improved technology. This process usually involves disassembling the device and analyzing its components and workings in detail, and this is precisely what biologists have been doing with living systems for the past century. But rather than trying to identify key component parts or mechanisms (as is the main focus of biology), reverse engineering seeks to identify the minimal set of design principles that are necessary to reconstitute relevant functions of the whole.

Virtually all organs contain an interface between a parenchymal tissue (e.g., epithelium, connective tissue, nerve tissue) and a vascular tissue (blood vessel) that perfuses the organ with crucial oxygen and nutrients, delivers immune cells, and transfers chemical, molecular, and hormonal signals from one organ to another. All organs also experience characteristic mechanical forces (e.g., cyclic strain, compression, shear) as well as neuroelectrical cues that are crucial for normal organ physiology. Thus, if one were to reverse engineer an organ, it would be critical to reconstitute characteristic tissue-tissue interfaces, ensure vascular perfusion, and provide an organ-relevant physical microenvironment. These are the precise minimal design principles that inspired the development of "organs-on-chips," which now permit in vitro analysis of how cells collaborate within human tissues and organs to regulate normal physiology, as well as promote disease development.

Organs-on-Chips

Organs-on-chips are microfluidic cell culture devices created with computer microchip manufacturing techniques that contain hollow microchannels lined by

living cells and tissues cultured within an organ-relevant physical context, which are continuously perfused with life-sustaining culture medium (Bhatia and Ingber, 2014). This field has now advanced to the point where it is possible to engineer microfluidic organs-on-chips that contain complex, organ-specific, tissue-tissue interfaces (e.g., alveolar-capillary interface) by culturing different cell types on the opposite sides of a common extracellular matrix (ECM)-coated, porous membrane that separates two, independently perfused, parallel microchannels within a single microfluidic device (Huh et al., 2010; Achyuta et al., 2013; Kim et al., 2016; Benam et al., 2016). Similar tissue-tissue interfaces have been created using a viscous fingering technique to form a cylindrical ECM gel within a microfluidic channel containing a central lumen that can be lined by one type of tissue (e.g., epithelium) while culturing another cell type (e.g., fibroblasts) in the surrounding gel (Bischel et al., 2015; Herland et al., 2016). Relevant mechanical forces can be applied to recreate physiological movements (Huh et al., 2010; Kim et al., 2016) and fluid shear stresses (Bhatia and Ingber, 2014; Achyuta et al., 2013; Kim et al., 2016; Benam et al., 2016; Bischel et al., 2015; Herland et al., 2016); air-liquid interfaces can be recreated by introducing air into the parenchymal tissue channel (Huh et al., 2010; Benam et al., 2016); and electrical signals can even be applied by integrating electrodes into the devices (Douville et al., 2010). Additional tissue types also can be integrated into the system, including circulating immune cells (Huh et al., 2010; Kim et al., 2016; Benam et al., 2016), connective tissue cells (Bischel et al., 2015), neuronal cells (Achyuta et al., 2013), and even living microbiome (Kim et al., 2016) to study more complex tissue-tissue interactions and more faithfully mimic complex organ-level responses. The chips may be populated with primary human cells, cell lines, stem cells or inducible pluripotent stem cells from healthy or diseased human patients. Finally, the vascular channels of different organ chips can be linked fluidically to study higher order, multi-organ physiological coupling (Maschmeyer et al., 2015; Esch et al., 2014). And all of this can be done while carrying out high-resolution, real-time imaging, and in vitro analysis of biochemical, genetic, and metabolic responses.

Intercellular communication in a human organ context in vitro

Microfluidic devices have been used for many years to probe cell-cell interactions; to explore the effects of chemical or cytokine gradients on cell behavior; and to study the effects of drugs, toxins, and mechanical forces on cultured cells and tissues in vitro (Bhatia and Ingber, 2014). More recently, however, organs-on-chips that incorporate more complex tissue-tissue interfaces have begun to be used to study how cells within different tissues exchange molecular signals, collaborate, and respond to physical cues to regulate complex organ-level physiology and contribute to disease development.

Blood-Brain Barrier

For example, a blood-brain-barrier (BBB)-on-a-chip has been developed and used to analyze intercellular signaling that mediates neuroinflammation. Rat brain microvascular endothelial cells are cultured in a microchannel on one side of a porous membrane, and a mixture of brain neurons and supporting astrocytes and microglial cells are grown on the other to recreate the neurovascular unit (Achyuta et al., 2013). Addition of the inflammatory cytokine, TNF-α, to the vascular channel results in increased expression of the vascular adhesion molecule ICAM-1 on the surface of the neighboring endothelium as well as concomitant activation of the adjacent microglia and astrocytes (as indicated by upregulation of GFAP), much as is observed in neuroinfectious diseases in vivo. Use of this microengineered BBB model reveals that this response is mediated by the intact endothelium and is not merely due to a leaky vasculature. The neuronal cells also produce detectable inhibitory and exciting potentials, and thus, contributions of electrical signals to normal BBB function could be studied using this model in the future.

More recently, neuroinflammation has been analyzed using a 3D microfluidic model of an all human BBB composed of a hollow cylindrical ECM gel filled with astrocytes and a central lumen lined by brain microvascular endothelium and circumferential pericytes (Herland et al., 2016), which closely mimicks the natural BBB. Stimulation of this endothelium with TNF-α results in unique expression profiles of the inflammatory cytokines G-CSF and IL-6 that mediate neuroprotection and neuroactivation in vivo, depending on whether pericytes or astrocytes are present in the surrounding ECM, and these profiles are different than those expressed by cells grown in static Transwell co-cultures.

Lung

Studies using a human lung-on-a-chip that reconstitutes the alveolar-capillary interface demonstrates that the addition of either TNF-α, simulants of airborne pollutants (12 nm silica nanoparticles), or living bacteria to the epithelial space not only induces expression of ICAM-1 in the underlying endothelium, it also results in enhanced recruitment of circulating human neutrophils when they are perfused through the vascular channel (Huh et al., 2010). Due to their high visual clarity, these devices enable high-resolution microscopic analysis of the multicellular interactions that mediate human lung inflammation. In addition, by applying cyclic suction to microchambers that run parallel to the culture channels, it is possible to mimic normal breathing motions in this lung chip by rhythmically stretching and relaxing the porous membrane and adherent tissue layers. Interestingly, neither epithelial injury nor activation of the endothelium and the downstream inflammatory response are observed in the absence of physiological breathing motions. This ability to analyze physiological responses at the organ level also resulted in the discovery that transcytosis of the nanoparticles across both the epithelial and endothelial tissue layers, and their resulting absorption into the vascular channel, are similarly sensitive to cyclic mechanical cues, and again this response is not observed in static chips or Transwell co-cultures. Importantly, this previously unknown mechanosensitive signaling response has been confirmed to be physiologically relevant in that it also is observed in whole lung when similar studies are carried out in an ex vivo mouse ventilation-perfusion model.

Thus, use of this lung alveolus-on-a-chip has uncovered previously unknown