A Human Breathing Lung-on-a-Chip

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Abstract

Here we describe a microphysiological system that replicates the functional unit of the living human lung. This human “breathing lung-on-a-chip” microdevice provides unique capabilities to reconstitute three-dimensional microarchitecture, dynamic

mechanical activity, and integrated physiological function of the alveolar–capillary interface. We demonstrate the potential of this microengineered biomimetic model for screening environmental particulates and modeling complex human disease processes.

Keywords: organ-on-a-chip; microfluidics; lung; lung-on-a-chip

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Current research efforts in drug development and toxicology are often challenged by a lack of predictive models to reliably assess physiological responses of the human body to drug compounds and toxins. Despite the development of various types of surrogate models based on *in vitro* cell culture techniques, the vast majority of these systems fail to faithfully recapitulate the structural and functional complexity of living tissues and organs. Although three-dimensional extracellular matrices have been used to induce differentiated tissue-specific phenotypes (1), this approach is still limited in its ability to emulate the essential architecture and spatial arrangements of multiple tissue types that are central to various physiological functions. Existing cell culture models also lack the ability to reproduce complex, dynamic mechanical and biochemical microenvironments that play a critical role in organ development and function (2).

Here we introduce the proof-of-concept of leveraging microscale engineering technologies to develop microengineered biomimetic microsystems that recapitulate the structural, environmental, and functional complexity of living human organs

(development and application of the lung-on-a-chip model have been published previously [3]). Specifically, we created a microfluidic device that replicated the microarchitecture and dynamic microenvironment of the alveolar–capillary unit of the living human lung (3–6). To develop this microengineered lung model, we used soft lithography–based microfabrication techniques to construct a compartmentalized three-dimensional microchannel system consisting of the upper and lower cell culture chambers separated by a 10- μ m-thick microporous elastomeric membrane made of poly (dimethylsiloxane). For the formation of an alveolar–capillary interface, human alveolar epithelial cells and pulmonary microvascular endothelial cells were seeded into the upper and lower chambers, respectively, and allowed to adhere to extracellular matrix–coated membrane surfaces. Once cell attachment was established, the microchannels were perfused with culture media to support cell growth to confluence. Subsequently, the alveolar epithelial cells were exposed to air to induce their differentiation while being fed from the basal side, and this culture condition led to the formation of a microengineered

barrier tissue composed of the alveolar epithelium and capillary endothelium in close apposition (Figures 1A and 1B). In addition, cyclic vacuum suction was applied to the side hollow chambers adjacent to the cell culture channels to induce cyclic mechanical stretching of the adherent cell layers that simulated physiological breathing motions and resultant deformation of the alveolar–capillary barrier (Figure 1A).

Importantly, this lung-on-a-chip model provides new opportunities to reconstitute organ-level physiological functions that arise from complex interplay between multiple tissue types in the living human lung. For example, introduction of inflammatory insults, such as proinflammatory cytokines and bacteria, into the upper alveolar compartment induced activation of the endothelial cells on the opposite side of the membrane and their increased expression of adhesion molecules. This response was followed by adhesion of human neutrophils circulating in the lower vascular channel to the endothelium and subsequent transmigration across the tissue layers into the airspace (Figure 1C). The neutrophils then underwent directional migration toward

Figure 1. A human breathing lung-on-a-chip. (A) The microfabricated lung mimic device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the poly(dimethylsiloxane) membrane forming the alveolar–capillary barrier. (B) Long-term microfluidic co-culture produces a tissue–tissue interface consisting of a single layer of the alveolar epithelium (Epi; green) closely apposed to a monolayer of the microvascular endothelium (Endo; red), both of which express intercellular junctional structures such as occludin or vascular endothelial (VE)-cadherin. (C) Neutrophils flowing in the lower vascular channel adhere to the endothelium activated by *Escherichia coli* in the alveolar chamber, transmigrate (top row; the yellow arrow shows the direction of cell migration), emigrate into the alveolar space (middle row; the yellow arrow shows a transmigrating neutrophil), and engulf the bacteria (bottom row). Adapted from Figures 1, 2, and 3 in Reference 3.

the bacteria and engulfed them. As demonstrated in this study, optical transparency of poly(dimethylsiloxane) makes it possible to directly observe and analyze the entire process of complex physiological responses in real time.

Using the lung-on-a-chip microdevice, we conducted nanotoxicology studies to examine

production of intracellular reactive oxygen species in response to alveolar exposure to nanoparticles. In this investigation, our model revealed induction and amplification of acute toxic responses to silica nanoparticles with a diameter of 12 nm due to cyclic stretching of the alveolar–capillary barrier that simulated physiological

breathing motions (Figure 2A). Moreover, breathing-induced physiological mechanical forces also increased absorption of the silica nanoparticles from the airspace to the microvascular channel (Figure 2D). These key findings in our microfluidic system were reproduced in animal studies using a whole mouse lung

Figure 2. Microengineered model of pulmonary nanotoxicology. (A) Alveolar epithelial cells increase reactive oxygen species (ROS) production when exposed to ultrafine silica nanoparticles in conjunction with 10% cyclic strain, whereas nanoparticles or strain alone had no effect relative to control cells (circles). (B) Transport of nanomaterials across the alveolar–capillary interface is simulated in the lung mimic device. (C) 10% mechanical strain significantly increased the rate of nanoparticle translocation compared with static controls in this device or in a Transwell culture system. (D) Fluorescence micrographs of a histological section of the whole lung showing 20-nm fluorescent nanoparticles (white dots, indicated with arrows) after intratracheal injection of nebulized nanoparticles and ex vivo ventilation in the mouse lung model. Nanoparticles cross the alveolar–capillary interface and are found on the alveolar epithelium, in the interstitial space, and on the capillary endothelium (PC, pulmonary capillary; AS, alveolar space; blue, epithelial nucleus; bar, 20 µm). The graph shows that physiological breathing in whole mouse lung produces more than a fivefold increase in nanoparticle absorption into the blood perfusate when compared with lungs without lung ventilation. Adapted from Figure 4 in Reference 3.

Figure 3. Microengineered model of (A) pulmonary edema that reconstitutes (B) fluid accumulation and (C) deposition of fibrin clots in the alveolar airspace. Adapted from Figure 1 in Reference 7.

ventilation-perfusion model, demonstrating physiological relevance of the lung-on-a-chip model.

Finally, we leveraged this device to develop a microengineered model of pulmonary edema in human lungs (7). Specifically, our disease model was designed to simulate the development of vascular leakage syndrome and resultant pulmonary edema in patients with cancer who undergo chemotherapy using interleukin-2 (Figure 3) (8). Administration of interleukin-2 into the vascular channel of the lung-on-a-chip device resulted in leakage of culture medium from the vascular compartment and concomitant fluid accumulation in

the upper alveolar channel. This injurious response was sustained continuously over a period of 4 days until the airspace became completely flooded (Figure 3A). This model also replicated deposition of fibrin clots in the alveolar compartment due to enzymatic reactions between plasma proteins during the progression and exacerbation of pulmonary edema (Figure 3C). Furthermore, our study suggested previously unknown adverse effects of physiological mechanical forces on interleukin-2-induced increases in the permeability of the alveolar-capillary barrier.

The human breathing lung-on-a-chip microdevice demonstrates a proof-of-principle for integrating

microfluidics and microfabrication technologies with cultured living cells to reconstitute the most salient structure, environment, and function of living human organs. This organ-on-a-chip approach represents a major advance from traditional cell culture and is readily applicable to developing physiological models of other organs and organ systems in the body. We believe that these biomimetic microsystems hold tremendous potential as an innovative and predictive screening platform for a variety of biomedical, pharmaceutical, and environmental applications. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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