Lung Organomimetic Microdevice

Dongeun Huh[1,2] and Donald E. Ingber[1,2,3]

[1]Vascular Biology Program, Departments of Pathology and Surgery, Harvard Medical School and Children's Hospital Boston, USA

[2]Wyss Institute for Biologically Inspired Engineering, Harvard University, USA

[3]School of Engineering and Applied Sciences, Harvard University, USA

ABSTRACT

Pulmonary alveoli play a vital role in the maintenance of normal physiological function of the lung, as well as in the pathogenesis and progression of various pulmonary diseases. Because of the complex architecture of the lung, the small size of lung alveoli, and the dynamic mechanical motions of this organ, it is difficult to study this structure in whole animals at the microscale. Here we describe a novel organomimetic microsystem that enables on-chip generation of functional alveolar epithelial tissues and reproduction of their dynamic mechanical microenvironment *in vitro*. This microengineered system represents a first step towards the development of more sophisticated *in vitro* models of complex structure and physiology of the living lung.

KEYWORDS: Lung, Microfluidics, Cell, Organ

INTRODUCTION

The alveolus is the most important functional unit of the lung, as it is where gas exchange and absorption of airborne particles and chemicals occur. The alveolus consists of a single layer of alveolar epithelial cells and a second layer of pulmonary endothelium separated by a thin extracellular matrix. Despite the vital role of the alveolar system in health and disease, much remains to be learned about the interplay between its cellular constituents and their mechanical microenvironment in the maintenance of normal physiology, as well as in the development of pulmonary diseases. This is mainly due to challenges associated with developing experimental models that recapitulate the key structures, physiological functions, and dynamic mechanical motions of the lung. Here we describe a mechanically-actuatable organomimetic microsystem that can mimic integrated structure and functions of the alveolar system through on-chip engineering of alveolar epithelial cells and reproduction of physiological mechanical forces generated by breathing.

EXPERIMENTAL

The microengineered lung mimetic consists of two adjacent microchannels separated by a thin flexible membrane fabricated in poly(dimethylsiloxane) (PDMS) using a new method based on selective chemical etching [1] of PDMS microstructures (Figure 1). Alveolar epithelial cells are seeded into the upper culture channel and grown on the membrane. For cell stretching, vacuum is applied to side chambers to induce elastic deformation of the membrane; this causes membrane stretching and strain application to the adherent cell layer (Figure 2). By using a computer-

978-0-9798064-2-1/μTAS2009/$20

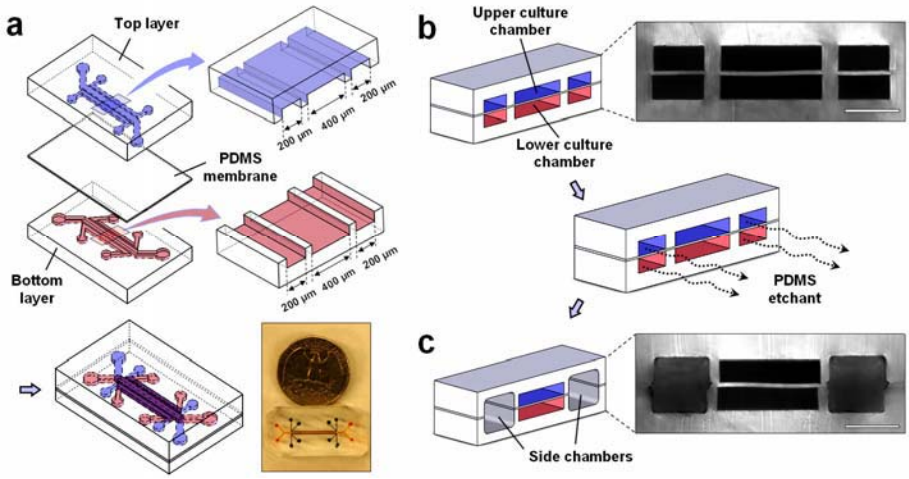

Figure 1. a, The microfluidic device consists of top and bottom layers separated by a 10 μm-thick PDMS membrane. **b,** Two sets of the three parallel microchannels in the top and bottom layers are aligned and permanently bonded. Subsequently, a PDMS etching solution is introduced into the side channels in the top and bottom layers. **c,** The flow of PDMS etchant etches away PDMS membrane segments in the side channels, resulting in the generation of two side chambers. Scale bars, 200 μm.

controlled vacuum system integrated with the microfluidic device, it is possible to manipulate strain and stretching frequency, and thereby recreate the physiological microenvironment experienced by lung cells during breathing.

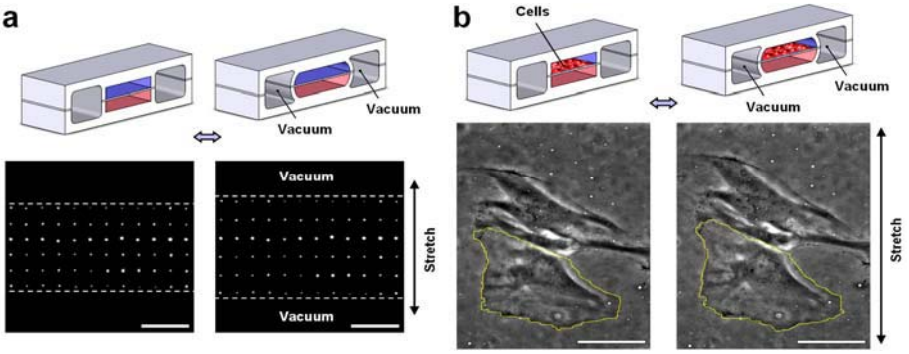

Figure 2. a, A PDMS membrane is patterned with an array of 10-μm fluorescent quantum dots and embedded in the device. Vacuum applied to the side chambers causes membrane stretching, as visualized by changes in the position of the fluorescent dots (top-down view). Scale bars, 200 μm. **b,** Living cells attached to the PDMS membrane become stretched and increase their projected surface area in response to membrane stretching. Scale bars, 150 μm.

RESULTS AND DISCUSSION

We first demonstrated microfluidic culture of alveolar epithelial cells for extended periods of time. Alveolar epithelial cells seeded in the upper microchannel attached to the membrane coated with fibronectin and grew to confluence over the period of three days (Figure 3a). The cells remained viable in the microfluidic device over two weeks with continuous delivery of culture medium by a syringe pump. We also confirmed that vacuum application resulted in controlled application of mechanical strain to the cells without causing cell detachment or loss of viability.

We then used the same microsystem to investigate the potential toxic effects of nanoparticulates on lung alveolar epithelial cells. Specifically, we measured the production of reactive oxygen species (ROS) in cells caused by different types of nanomaterials. ROS measurements were taken by labelling the epithelial cells with a ROS indicator dye and detecting increases in intracellular fluorescence in response to nanoparticle addition.. Using this model, we showed that the cells increased production and accumulation of intracellular ROS when exposed to silica nanoparticles (12 nm in diameter) in combination with cyclic mechanical strain that mimicked physiological breathing (Figure 3b). We also detected larger numbers of nanomaterials inside the cells in the presence of cyclic strain. These results suggest that physiological mechanical strain can enhance nanoparticulate-induced oxidative stress and cytotoxicity by facilitating cellular uptake of these nanomaterials, and by causing an early increase in intracellular ROS.

CONCLUSIONS

Our lung mimetic system described here provides a multifunctional platform that enables analysis of various important but understudied areas of lung biology, as well as biological interfaces found in other physiological systems. This microengineered

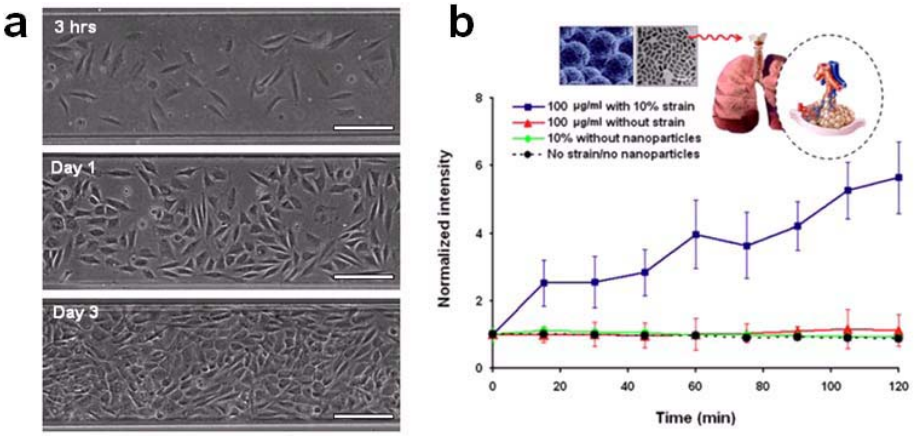

Figure 3. a, Pulmonary alveolar epithelial cells are seeded into the microfluidic channel and grown to confluence over the period of three days. **b,** The alveolar epithelial cells show a continuous increase in intracellular ROS when they are subjected to 12-nm silica nanoparticles along with physiological strain (blue). The cells, however, do not respond to the nanoparticles or mechanical strain alone as demonstrated by red and green lines.

alveolar system can be further improved to reconstitute complex structure and function of the living lung, by incorporating a living pulmonary capillary endothelium. We also believe that this type of bioinspired microsystem technology can be multiplexed and automated to provide high-throughput analysis for drug screening, toxicology, and bio-detection applications.

ACKNOWLEDGEMENTS

We thank A. Mammoto, M. Montoya-Zavala, and C. K. Thodeti for their assistance in cell culture, ROS assays, and fluorescence imaging. We also thank G. M. Whitesides, P. Cherukuri, B. Matthews, and N. Korin for their helpful comments and discussions, and R. Ruch for providing alveolar epithelial cells. D. H. is a recipient of a Wyss Technology Development Fellowship from the Wyss Institute for Biologically Inspired Engineering at Harvard University.

REFERENCES

[1] S, Takayama, E. Ostuni, X. P. Qian, J. C. McDoanld, X. Y. Jiang, P. LeDuc, M. H. Wu, D. E. Ingber, and G. M. Whitesides, Topographical micropatterning of poly(dimethylsiloxane) using laminar flows of liquids in capillaries, Advanced Materials, Vol. 13, pp. 570-574 (2001).