A MICROFLUIDIC ARRAY OF CYCLICALLY STRETCHABLE LUNG AIR-BLOOD BARRIERS

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ABSTRACT

We present a new biomimetic air-blood barrier that can cyclically be stretched in three dimensions by means of a "microdiaphragm". This lung-on-chip is made of two reversibly bonded parts allowing for an accurate control of the density of cells seeded on both sides of a thin, flexible and porous membrane. Co-cultures of lung epithelial and endothelial cells could successfully be established. Preliminary stretching assay showed that the mechanical strain importantly affects the barrier permeability. The system that contains an array of three air-blood barriers placed in parallel could easily be expanded in view of higher throughput applications.

KEYWORDS: Lung–on-chip, air-blood barrier, mechanobiology, biomimetic model, array

INTRODUCTION

Microfluidic in-vitro models of human organs offer unique possibilities to study biological processes in in-vivo-like conditions and are widely seen as having the potential to reduce animal testing for toxicology and for drug discovery applications. In sharp contrast to commercially available in-vitro models, based on Petri dishes, those models enable the reproduction of key biophysical parameters, such as the cyclic mechanical strain induced by the breathing motion. Recently, Huh and colleagues [1] reported about a microfluidic in-vitro model, capable of reproducing the cyclic stretching taking place in the lung alveoli. In contrast to that model, we present a system that mimics the mechanical strain taking place in the lung parenchyma (three-dimensional strain vs. one dimensional strain) in an unprecedented way. In addition, the handling of the device is made very simple due to a reversible bonding strategy that enables the accurate control of the cell seeding density on both sides of the air-blood barriers.

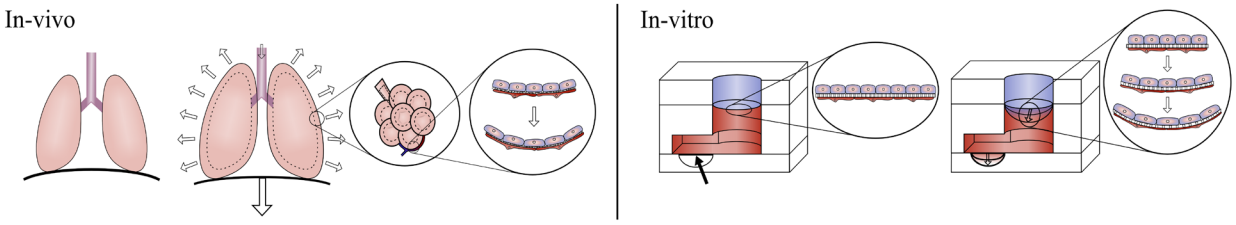

Figure 1: Illustration of the respiratory movements of the lung (left) and the actuation principle of the new in-vitro model (right). During respiration the diaphragm contracts, the lung expands, which induces a 3D stretching of the air-blood barrier. In the in-vitro model the air-blood barrier is stretched in three dimensions by controlling the deflection of an microdiaphragm (black arrow).

EXPERIMENTAL

The microfluidic air-blood barrier array consists of two parts made of PDMS: (i) a fluidic and (ii) a pneumatic part (Fig. 2 A). The fluidic part consists of a thin, flexible and porous membrane sandwiched between two microstructured elements (top plate and middle plate), whereas the pneumatic part is made of a non-porous membrane (actuation membrane = microdiaphragm) bonded by plasma oxygen on a bottom plate in which microchannels are structured. The device is fabricated by PDMS rapid prototyping. The rapid prototyping masters are produced in hard plastic by stereolithography (Proform AG, Marly, Switzerland). The thin porous membrane is fabricated by patterning an uncured, spin coated PDMS layer with a Si mold containing an array of micropillars produced by DRIE. The micropillars have a diameter of either 3um or 8um. The actuation membrane, is fabricated by spin coating PDMS on a Si wafer silanized with fluorosilane. Finally, the top plate of the fluidic part is plasma bonded on the thin porous membrane plasma bonded on the middle plate.

Prior to the cell culture experiments, the microfluidic device was first sterilized using an ozone cleaner (CoolCLAVE™, AMS Biotechnology, Switzerland). Then, the thin porous membrane was coated with human fibronectin (2.5 µg/cm^2). Human umbilical vein endothelial cells (HUVEC, 1x10^4 cells/cm2) were seeded on the basal side of the membrane and allowed to adhere for 24h. The next day, human bronchial epithelial cells (16HBE14o-, 2.5x10^5 cells/cm2) were seeded on the apical side of the membrane. Upon confluency, after 3-4 days, the cells were stained using standard immunofluorescence protocols and the barrier integrity was analyzed by laser scanning microscopy (LSM710, Zeiss, Germany).

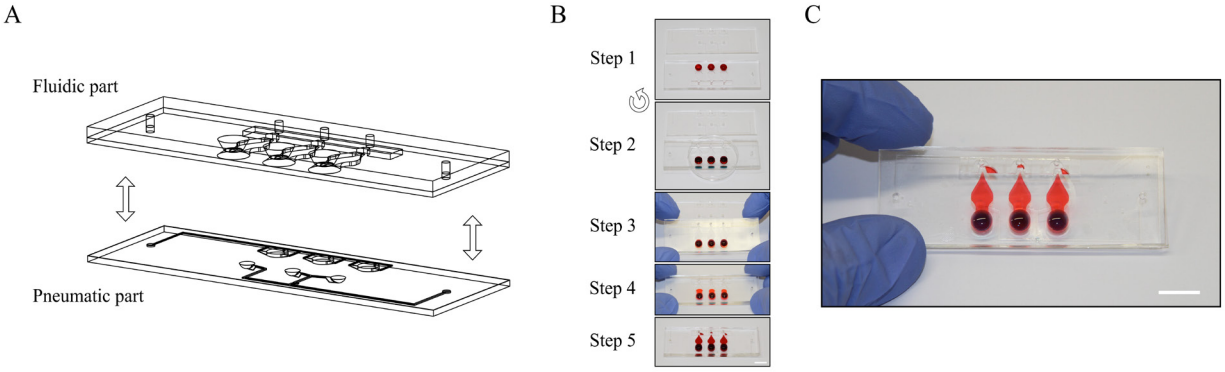

Figure 2: Illustration and photographs of the microfluidic array and its handling. (A) The fluidic and pneumatic parts are assembled reversibly. (B) This design enables a simple cell handling, where endothelial and epithelial cells can be cultured on both sides of the thin porous membrane. (C) After reaching confluence, the two parts are assembled and the cells are ready to be cyclically stretched. Scale bars 10 mm.

RESULTS AND DISCUSSION

Figure 1 shows the principle of the physiological respiratory movements, reproduced in the new microfluidic air-blood barrier model. Similarly to the in-vivo situation, the stretching of the in-vitro air-blood barrier is controlled via an actuation membrane (microdiaphragm). The compartment between the air-blood barrier and the actuation membrane is filled with medium. Therefore, the air-blood barrier follows the movement of the actuation membrane following Pascal's law. As a result the air-blood barrier is stretched in three dimensions, as it is the case in-vivo. The maximal strain applied to the cells is defined by the volume beneath the microdiaphragm.

The second key innovation of this device, is the reversible bonding between the fluidic and the pneumatic parts (figure 2 A). Figure 2 B shows how cells are cultured on chip. The cell culture method is similar to that used for transwell inserts. First the endothelial cells are seeded and cultured on the basolateral side of the porous membrane. After cell adherence, the fluidic part, housing an array of three air-blood barriers, is flipped and the epithelial cells are cultured on the apical side of the membrane. Once the cells are

confluent, a cyclic mechanical strain is applied to the cells via the pneumatic microchannels integrated in the pneumatic part of the device. The external electro-pneumatic setup controls the negative pressure applied to the actuation membrane.

The thin, porous and flexible membranes were produced at different thicknesses (3.5 um up to 10 µm), pore densities (60'000 – 800'000 /cm^2) as well as pore diameters (3 or 8 µm) (figure 3 A, B). Then lung epithelial (16HBE14o-) and blood vessel cells (HUVEC) were cultured on the fibronectin coated porous membrane to build an in-vitro air-blood barrier. Figure 3 D shows fluorescence micrographs of such an in-vitro barrier with endothelial cells reaching to the epithelial cells through the pores of the membrane. A 10 µm thin membrane with 8 µm pores was used in for the co-culture. Preliminary stretching experiments at physiological stress levels were promising and could be performed during several days (not shown). First results reveal that a physiological strain importantly affects the air-blood barrier permeability when a small molecule (FITC: Fluorescein isothiocyanate) is exposed to a lung epithelial cell barrier (16HBE14o-) (figure 3C). In contrast, the permeability of a larger molecule (RITC: Rhodamine B isothiocyanate) is not affect by the mechanical strain.

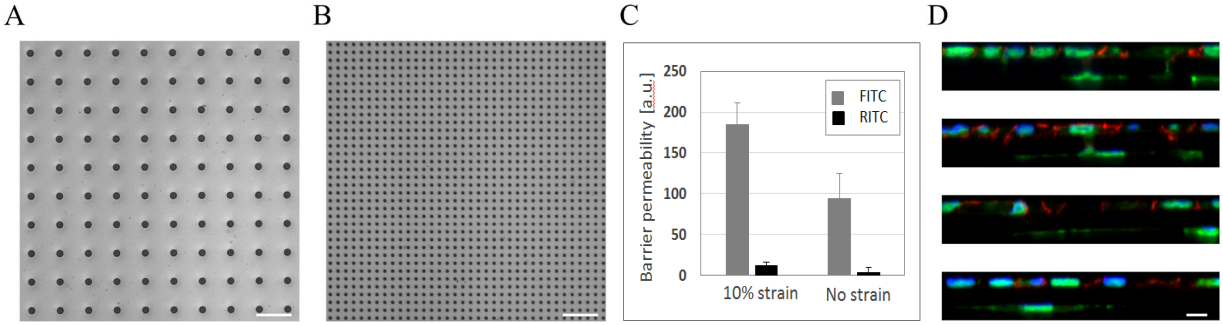

Figure 3: Micrographs of the porous and flexible membrane with 8um (A) and with 3um pores (B). (C) Permeability assay performed with two molecules (FITC and RITC) with and without strain. (D) Fluorescence micrographs of a co-culture of epithelial (16HBE14o-) and endothelial cells (HUVEC) on a porous membrane. Blue represents the nucleus (Hoechst), red the adherence-junctions of the epithelial (E-Cadherin) and green the junctions of the endothelial cells (VE-Cadherin). Scale bars, 50, 50 and 10 µm.

CONCLUSION

We developed a new microfluidic air-blood barrier array, which mimics the three-dimensional strain acting on the lung alveoli in-vivo in an unprecedented way. The array that includes a thin, flexible and porous membrane, is simple to use thanks to the reversible bonding strategy, that enables to accurately control the cell seeding density. Preliminary stretching experiments reveal that a physiological strain importantly affects the air-blood barrier permeability depending on the size of the molecule.

ACKNOWLEDGEMENTS

We thank the Swiss Federal Laboratories for Materials Science (EMPA) for technical support and the Microscopy Imaging Center Bern (MIC) for the infrastructure. This work was supported by the Gebert Ruef foundation, the Swiss Commission for Technology and Innovation (CTI) and the Lungenliga Bern.

REFERENCES

[1] D. Huh, B. D. Matthews, a. Mammoto, M. Montoya-Zavala, H. Y. Hsin, and D. E. Ingber, "Reconstituting Organ-Level Lung Functions on a Chip," *Science*, vol. 328, no. 5986, pp. 1662–1668, Jun. 2010.

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