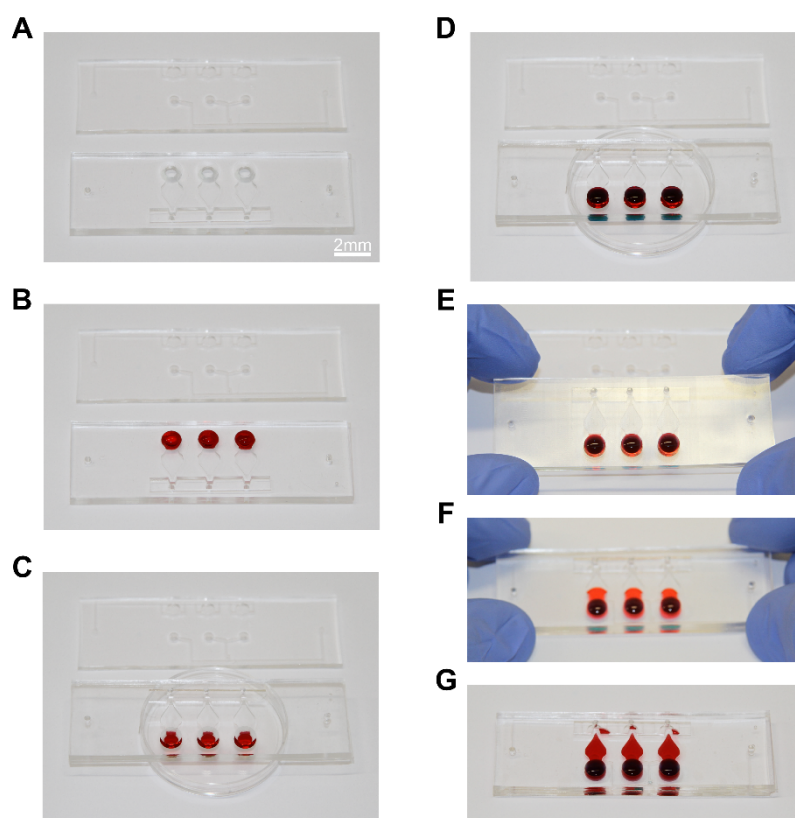


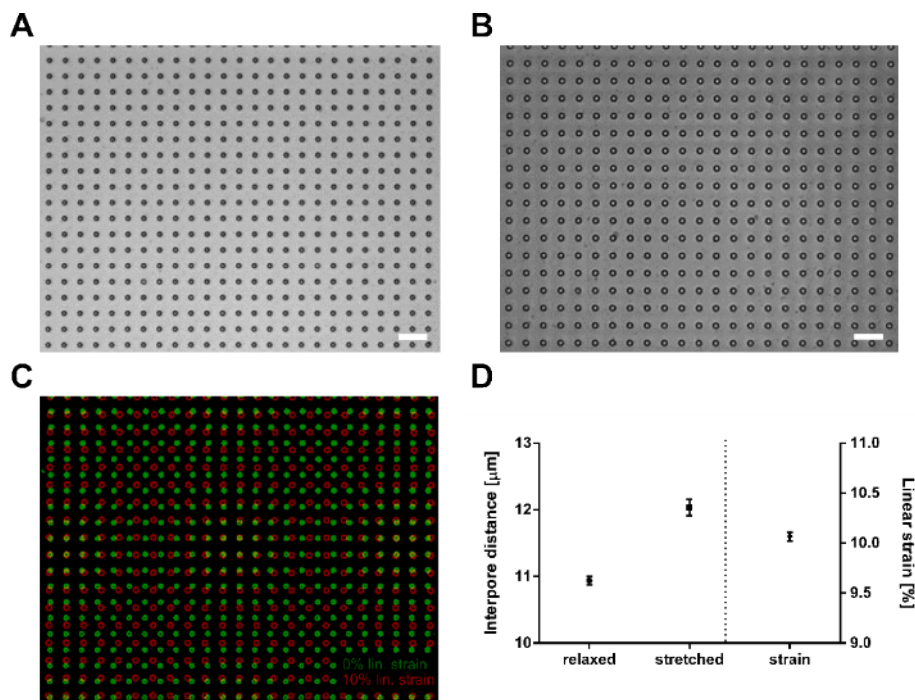
A lung-on-chip array with an integrated bio-inspired respiration mechanism

Supplementary information

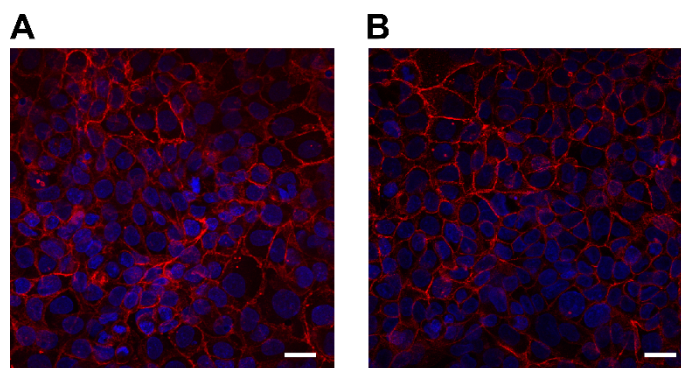
Supplementary Movie S1: Real-time stretching of primary human pulmonary alveolar epithelial cells on a $3\mu\text{m}$ porous, $3.5\mu\text{m}$ thin and flexible PDMS membrane at a frequency of 0.2 Hz.



Supplementary Figure 1. The preparation of the chip can easily be performed using an extended hanging drop technique. First, the fluidic and pneumatic parts are placed on a surface (A). Then, a drop of cell culture medium containing a known concentration of suspended endothelial cells is added on the basolateral side of the porous membrane (B). The design of the basolateral chamber as well as the hydrophobic surface of the PDMS keeps the drop stable. This way the cells can be cultured within the drop until they are confluent. The cell culture medium can be exchanged by simply aspirating the liquid from the drop and adding fresh media. Once the cells reach confluency the fluidic part is flipped (C) with the cell culturing medium in a hanging drop. Then, epithelial cells can be added on the other side of the porous membrane (D). When the cells are confluent, the fluidic part is then gently brought together with the pneumatic part. The hanging drops are then forced into the microstructures of the basolateral chamber (F-G). The solution in excess flows via a microvalve in the overflow chamber (G). The operation can obviously also be performed, by seeding first epithelial cells and then endothelial cells.



Supplementary Figure 2. The maximum strain in the alveolar membrane was evaluated by comparing the porous membrane at rest (A) and at maximal deflection of the micro-diaphragm (B) (scale bar 20 μm). The two images were then post-processed in Fiji to create an overlay (C) to visualize the strain pattern. The distance between two adjacent pores (inter-pore distance) was measured and averaged, and the resulting strain was then calculated (D) (data is shown as mean \pm SD). The strain was found to correspond to the maximal strain of 10% linear. A 3.5 μm thin membrane with 3 μm pores, without cells was used for this experiment.



Supplementary Figure 3. Immunofluorescence images of lung epithelial cells (16HBE14o-) taken after the permeability assay. (A) static and (B) dynamic condition. Blue represents the nuclei and red the adherence junctions (E-cadherin). Scale bar 50 μm .