Electronic Supplementary Material (ESI) for Lab on a Chip.
This journal is © The Royal Society of Chemistry 2017

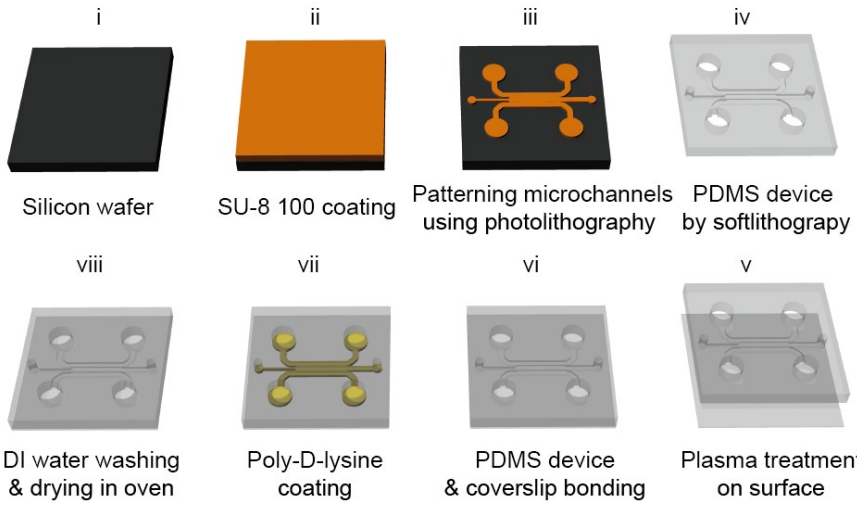

Figure S1. Fabrication and preparation of the PDMS micro-fluidic chip. (i-iii) By using a photolithography method, the molds of microchannels were patterned onto a photoresist-coated silicon wafer. (iv) The PDMS micro-fluidic chip was fabricated by curing a mixture of a PDMS base solution and curing agent (10:1, weight ratio) on the patterned wafer. (v-vi) The autoclaved PDMS chip and glass coverslips were bonded using oxygen plasma treatment on each surface. (vii) After bonding, 60 µl of poly-D-lysine (PDL) solution (1 mg/ml) was added into the microchannels and the chip was incubated at 37°C for 3 hours. (viii) The PDL coated chip was washed twice with deionized water and dried in an 80°C oven for 12 hours.

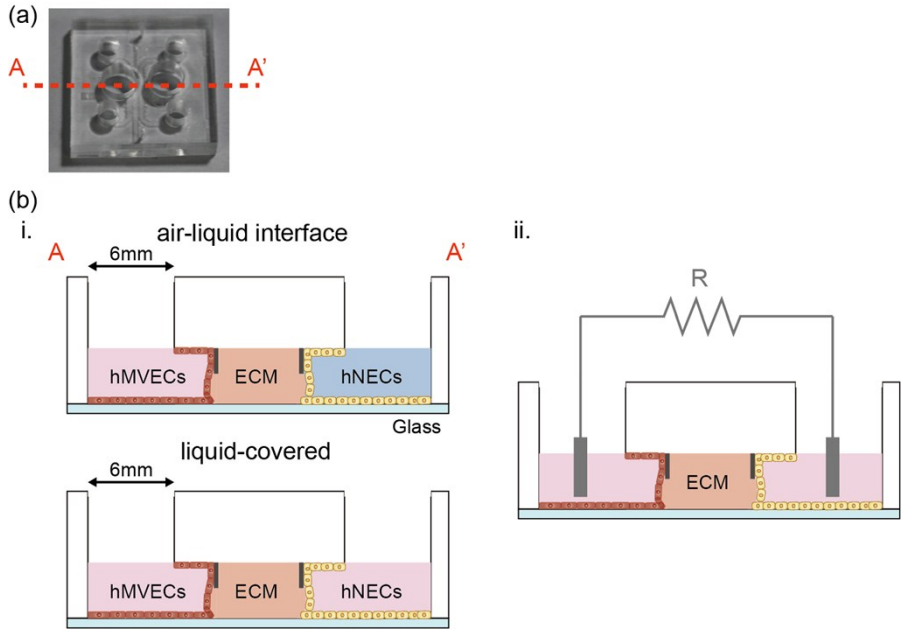

Figure S2. TEER measurement in our microfluidic device. (a) Image of modified device for TEER measurement. The holes which are 6 mm diameter each and partially overlapped cell channels were punched before glass bonding. (b) Schematic of TEER measurement. i. the hNECs were cultured and formed monolayer in two types of culture conditions (air-liquid interface, liquid-covered). ii. Shortly before measurement, the medium was filled into hNECs channel in air-liquid interface case for identical measurement environment. After measurement, the medium eliminated and hNECs were cultured in air-liquid interface as before.

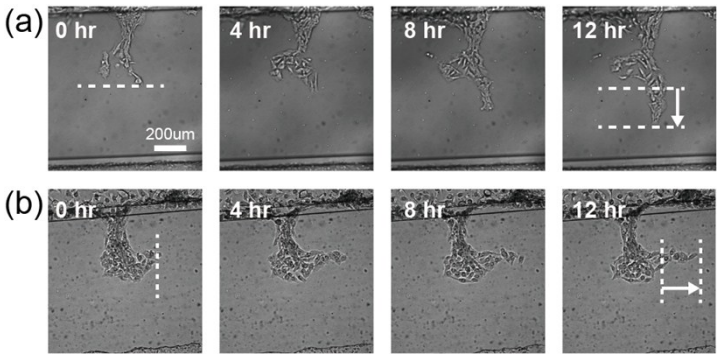

Figure S3. Time lapse images of the formation of gland-like structures. The development of gland-like structures in ECM could be categorized into two steps. (a) Length growth (vertical to hNECs layer), and (b) expansion of the front side of gland-like structures (horizontal to hNECs layer). The arrows indicate the direction of gland-like structure development. (scale bar: 200um)

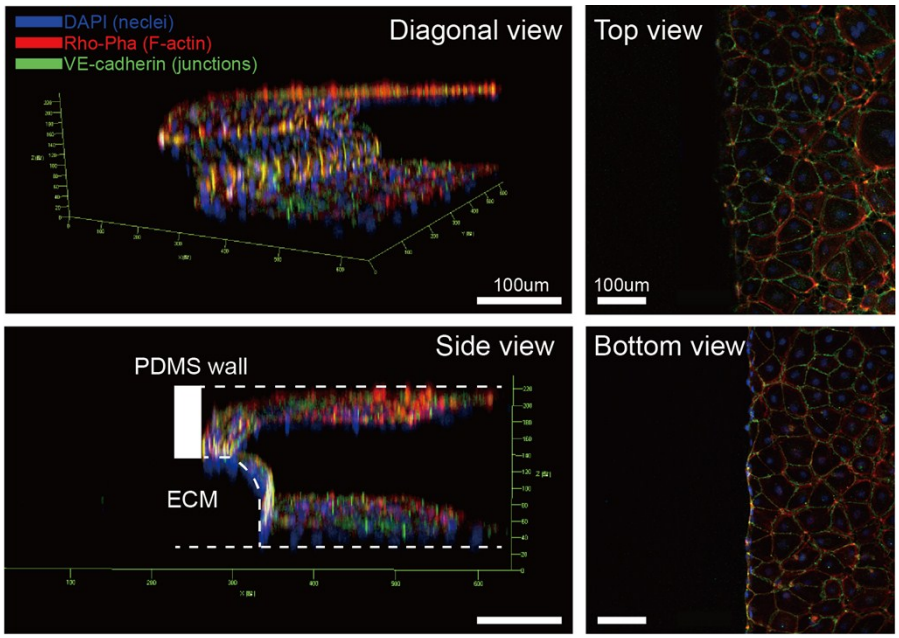

Figure S4. Fluorescence images of the hMVECs monolayer. Cells were stained with VE-cadherin (Green), Rhodamine-phalloidin (Red), and DAPI (Blue). Diagonal and side view images show confluent endothelial monolayer. Also, the expression of tight junction protein in top or bottom view images indicates the barrier formed tightly. (scale bar: 100μm)

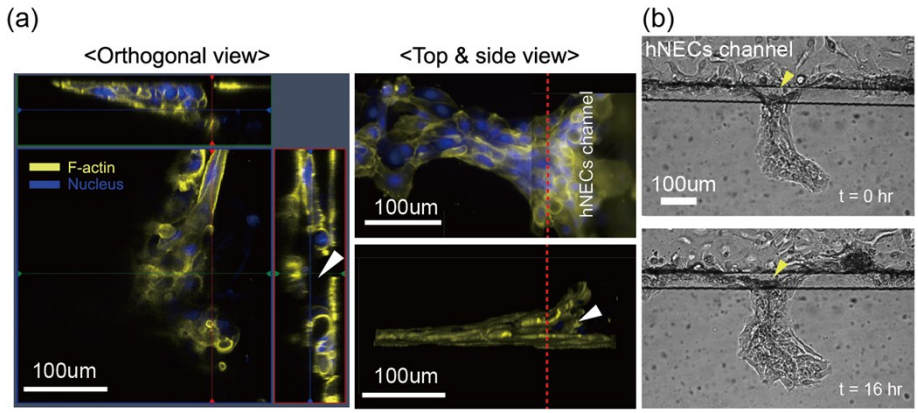

Figure S5. (a) The confocal images of gland-like structure in orthogonal, top, and side view. The lumen site at entrance region was successfully confirmed (white arrow). And, (b) time lapse images of epithelial layer. During gland-like structure formation, hNECs were migrated actively. But, the epithelial layer at entrance maintained lumen structure for this period (yellow arrow). (scale bar: 100μm)

Air-liquid interface culture type		Strong point	Weak point	Findings : achievements	Ref
In vitro	**Conventional product** (transwell insert, EpiAirway)	- **Easy cell culture** (cell seeding, medium exchange) - **Low cost** - **Form uniform and confluent epithelial barrier** - **Easy to apply convensional analysis methods** (qRT-PCR, Elisa, Western blot, staining etc.)	- **Simple 2D monolayer** (mono-cell culture, Cell-line based, or without ECM) - **Low physiological similarity** - **Short-term culture** (up to 1 weeks) - **Use artificial membrane** (Hard to morphogenesis or migration)	**Nasal epithelial barrier** : permeability, drug transport studies	**1**
				Lung epithelial barrier : permeability, response to air pollutant like diesel exhaust	**2**
				Bronchial epithelial barrier : permeability, cell layer morphology	**3**
				Bronchial epithelial barrier : permeability, drug transport study	**4**
				Bronchial epithelial barrier : expose smoking gas, compare to in vivo bronchial system (gene expression)	**5**
				Lung epithelial + Macrophage + dendritic cells : triple co-culture, response to gold nanoparticles	**6**
	Microfluidic device with thin membrane	- **Complicated model** (co-culture system, mimic cellular micro environment, incorporating ECM) - **Micro-scale structures** (similar to in vivo) - **Easy to modify design** (for modeling, drug screenin etc.)	- **Short-term culture** (up to 1~2 weeks) - **Hard to fabrication** (Chip fabrication, complicated assembly of multiple layers) - **Use artificial membrane** (Hard to morphogenesis or migration)	**Lung epithelium barrier** : permeability, function upon exposure to air	**7**
				Lung-on-a-chip : organ-level responses (ROS, permeability, cytokine response to inflammation, …) : Alveolar epithelium & endothelium co-culture system.	**8**
				Nasal epithelial barrier : FA toxicity test, using fibroblast cells (NIH/3T3) as feeder layer, live imaging	**9**
In vivo		- **Long-term** - **Complete micro environment**	- **High cost** **- Cannot isolate specific microenvironmental factor**	***In vivo* nasal** : Response to diesel exhaust particles (cytokines, chemokines)	**10** **11**
Hydrogel incorporating microfluidic device		- **No artificial membrane** (could induce and observe cell migration) - **Primary cell based co-culture system** - **Introducing gland-like structure** (Differentiation and morphogenesis from nasal epithelial cells)	- **Short-term culture** (up to 1 weeks) - **Need more factors for more *in-vivo* like model** (Fibroblast cells, multi-type of ECM, Nutrients, …)	**Human nasal epithelial and endothelial barrier** : Barrier permeability, optimum co-culture condition, Hypoxic condition, gland-like structure formation	

Table S1. Table for comparison with previous air-liquid interface culture methods.

Supplementary References

1 M.-K. Lee, J.-W. Yoo, H. Lin, Y.-S. Kim, D.-D. Kim, Y.-M. Choi, S.-K. Park, C.-H. Lee and H.-J. Roh, *Drug delivery*, 2005, **12**, 305–311.

2 J. Seagrave, S. Dunaway, J. D. McDonald, J. L. Mauderly, P. Hayden and C. Stidley, *Experimental Lung Research*, 2009, **33**, 27–51.

3 C. I. Grainger, L. L. Greenwell, D. J. Lockley, G. P. Martin and Ben Forbes, *Pharmaceutical research*, 2006, **23**, 1482–1490.

4 H. Lin, H. Li, H.-J. Cho, S. Bian, H.-J. Roh, M.-K. Lee, J. S. Kim, S.-J. Chung, C.-K. Shim and D.-D. Kim, *arXiv*, 2007, **96**, 341–350.

5 C. Mathis, C. Poussin and D. Weisensee, *American Journal of …*, 2013.

6 C. Brandenberger, B. Rothen-Rutishauser, C. Mühlfeld, O. Schmid, G. A. Ferron, K. L. Maier, P. Gehr and A. G. Lenz, *Toxicology and Applied Pharmacology*, 2010, **242**, 56–65.

7 D. D. Nalayanda, C. Puleo, W. B. Fulton, L. M. Sharpe, T.-H. Wang and F. Abdullah, *Biomed Microdevices*, 2009, **11**, 1081–1089.

8 D. Huh, B. D. Matthews and A. Mammoto, …, 2010.

9 W. Wang, Y. Yan, C. W. Li, H. M. Xia, S. S. Chao, D. Y. Wang and Z. P. Wang, *Lab on a chip*, 2014, **14**, 677–680.

10 D. Diaz-Sanchez, M. Jyrala, D. Ng, A. Nel and A. Saxon, *Clinical Immunology*, 2000, **97**, 140–145.

11 D. Diaz-Sanchez, A. Tsien and J. Fleming, *The Journal of …*, 1997.