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Supplementary material

Fabrication of Sac-like Hydrogel Membranes for Replicating Curved Tissue Barriers in Chips

Wenqi She,^a Chong Shen,^a Yinghua Ying^b and Qin Meng^{*a}

a Key Laboratory of Biomass Chemical Engineering (Education Ministry), College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, 310027, China

b Key Laboratory of Respiratory Disease of Zhejiang Province, Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, 310009, China

* Correspondence to: Dr. Qin Meng, College of Chemical and Biological Engineering, 13 Zhejiang University, 38 Zheda Road, Hangzhou, Zhejiang 310027, P.R. China. 14 <u>mengq@zju.edu.cn</u>



Fig. S1. Manufacture process (A) and actual image (B) of the microfluidic lung-on-a-chip. (Scale bar: 3 mm.)



Fig. S2. Process of cell seeding.



Fig. S3. Different combinational of hydrogels and pipes for the fabrication of sac-like membranes. A) Different sac-like hydrogel membranes varied in terms of materials. (Scale bar: 1 mm.) B-C) The top residual surface after hydrogel membranes were peeled away from PU pipe (B) and PE pipe (C) under optical microscopy. D) The differently scaled hydrogel membrane of 15 wt% F127-DA formed using PU pipes. (Scale bar: 3 mm.)



Fig. S4. Images of F127-DA hydrogel membranes dissolution in PBS at 4°C (A), 37°C (B), and 70°C (C) for 24 h. (Scale bar: 150 μm.)



Fig. S5. Phase contrast images of cells cultured on hydrogel membranes for 48 h prepared from pipes with different BP treatment times. (Scale bar: $100 \ \mu m$.)



Fig. S6. Flexibility and stability of F127-DA hydrogel membranes. A-B) The expansion (A) and contraction (B) process of sac-like hydrogel membranes in response to external pressure. C) The process of a hydrogel membrane from its dry state to its original form in an aqueous environment after storage in a refrigerator at 4°C for 3 months. (Scale bar: 500 µm.)



Fig. S7. CFD simulation of flow parameters in the liquid chamber. A) Relationship between the average wall shear force and inlet flow rate at different strains at 0-20%. B) Relationship between the maximum wall shear force and inlet flow rate at different strains at 0-20%. C) Relationship between the average flow rate in the liquid chamber and the inlet flow rate at different strains at 0-20%. D) Cloud diagrams of the wall shear force and the flow distribution in the liquid chamber at an inlet flow rate of 0.06 mL min⁻¹.



Fig. S8. Cells cultured on the hydrogel membrane. A-B) Quantification of membrane coverage by HPAEpiCs (A) and HUVECs (B) (referring to the percentage of the surface area covered by cells) for 5 days of culture at different seeding densities. Low density: 10^6 cells mL⁻¹, Medium density: 2×10^6 cells mL⁻¹, High density: 5×10^6 cells mL⁻¹. *p < 0.05, **p < 0.01, and ***p < 0.001. C) Cell viability of HPAEpiCs and HUVECs cultured for 7 days. D) Fluorescence images of HPAEpiCs and HUVECs cultured in the lung-on-a-chip at day 12. Green, live cells; red, dead cells. (Scale bar: 30μ m.)



Fig. S9. Comparison of HPAEpiCs and HUVECs performance in static and breathing culture. A) Mean fluorescence intensity (MFI) of EGFR, Vimentin, SPC, ZO-1 and F-actin expressed by HPAEpiCs. B) MFI of VE-cadherin, VEGF, ZO-1 and F-actin expressed by HUVECs. C-D) Confocal reconstruction view of the HPAEpiCs (C) and HUVECs (D) under static and 8 h of dynamic culture (cyclic strain at 10%, 0.2 Hz; flow rate at 0.06 mL·min⁻¹). Red, ZO-1; Green, F-actin; blue, nuclei. (Scale bar: 40 μm.)



Fig. S10. A-B) Secretion of Col-I(A) and IV (B) by HPAEpiCs under static and 8 h of dynamic culture on flat and sac-like F127-DA membrane. C-E) Secretion of ET-1 (C), ICAM-1 (D), and NO (E) by HUVECs under static and 8 h of dynamic culture on flat and sac-like F127-DA membrane. **p < 0.01 and ***p < 0.001.