Nanoscale

SUPPLEMENTARY INFORMATION

Large-scale fabrication of free-standing and sub-µm PDMS through-holes membranes

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SI1. Uniformity measurement of fabricated sub-µm photoresist column arrays

The uniformity of the sub-µm PR columns in each fabricated array was measured from the top-view HR-SEM images (Fig. S1). A high uniformity in the periodicity of the fabricated PR columns was obtained for all arrays. The flat-top diameter distribution of the PR columns analyzed by ImageJ software within the top-view HR-SEM images show a relatively small variation. This could probably come from the imperfection of the mask that was directly transferred to the patterned PR columns. In addition, the inaccuracy in the image processing and analysis using ImageJ software could also contribute to the errors.

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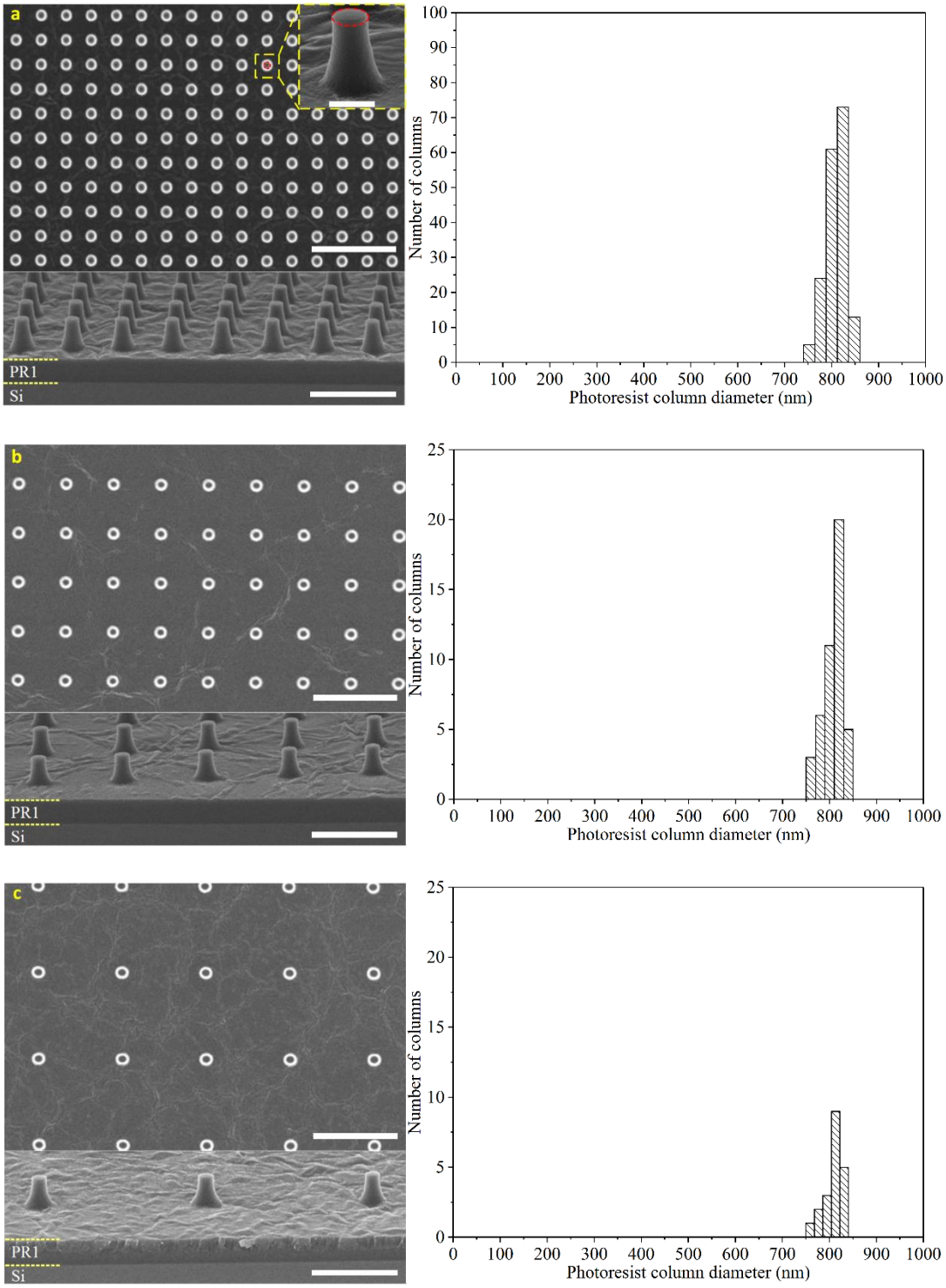

Fig. S1 Top-view (scale bar: 10 µm) and cross-sectional (scale bar: 5 µm) HR-SEM images of periodic sub-µm PR column with different pitches of a) 3 µm, b) 5 µm, and c) 10 µm, fabricated on the first, sacrificial, PR layer (PR1), and their corresponding flat-top diameter distribution. The black spots and the white rings in the top-view images indicate the flat-top and the sloped sidewall of the PR columns, respectively. The close-up image (scale bar: 1 µm) in a) shows the shape of a fabricated PR column, which has a sub-µm flat-top indicated by a red circle, and a sloped sidewall profile.

SI2. Spin-coating of PDMS:hexane solution over sub-µm photoresist column arrays

Fig. S2d-f show an atomic force microscope (AFM) image and the corresponding HR-SEM image of the surface of a PDMS membrane coated on a sub-µm PR column array, respectively. A low surface roughness (Ra) of 0.45 nm over a scan field of 1×1 μm^2 indicated a smooth membrane surface after the spin-coating and curing processes. The close-up HR-SEM image in Fig. S2f again confirmed the smoothness of the membrane surface.

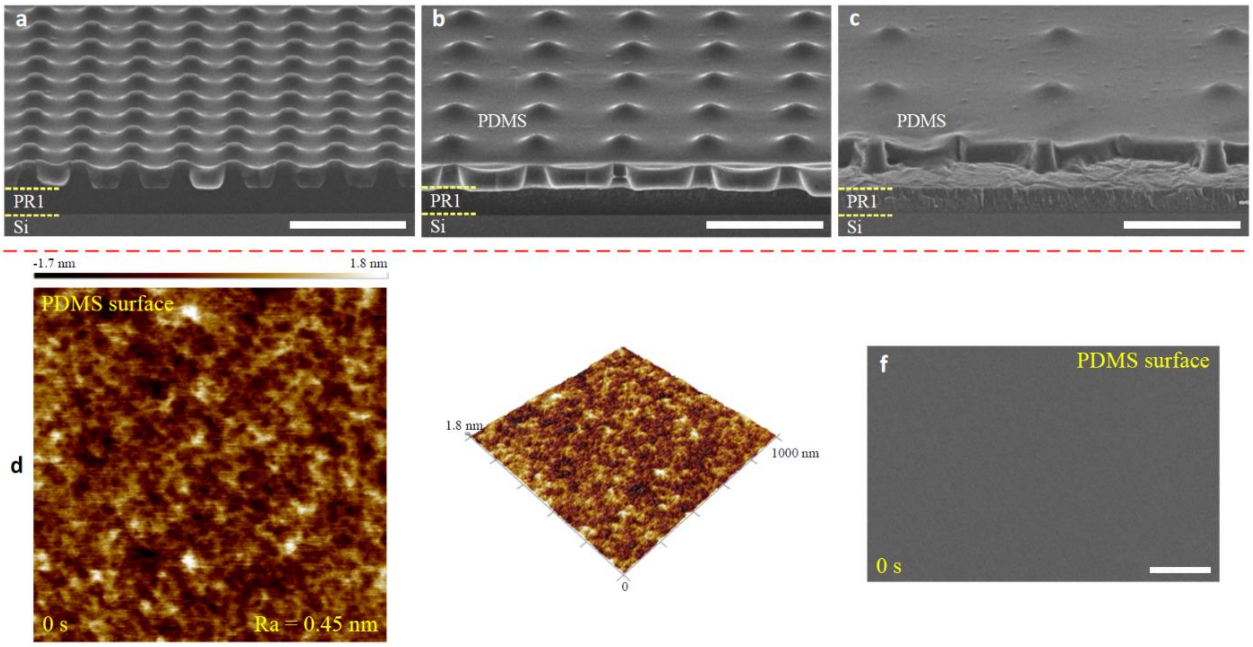

Fig. S2 Cross-sectional HR-SEM images (scale bar: 5 µm) of PR column arrays with different pitches of a) 3 µm, b) 5 µm, and c) 10 µm, coated with the cured PDMS:hexane (1:10) solution. d) An AFM image (scan field: 1×1 μm^2) of the surface of a spin-coated PDMS membrane, and f) a corresponding top-view HR-SEM image (scale bar: 200 nm).

SI3. Reactive plasma etching of PDMS

Fig. S3 shows the cross-sectional HR-SEM images of a PDMS-coated PR column array with the pitch of 5 µm, after etching for 30 s. The close-up HR-SEM image shows the peeling off and the stretching of the membrane (~960 nm thick) caused by breaking the sample, thus resulting in larger through-holes.

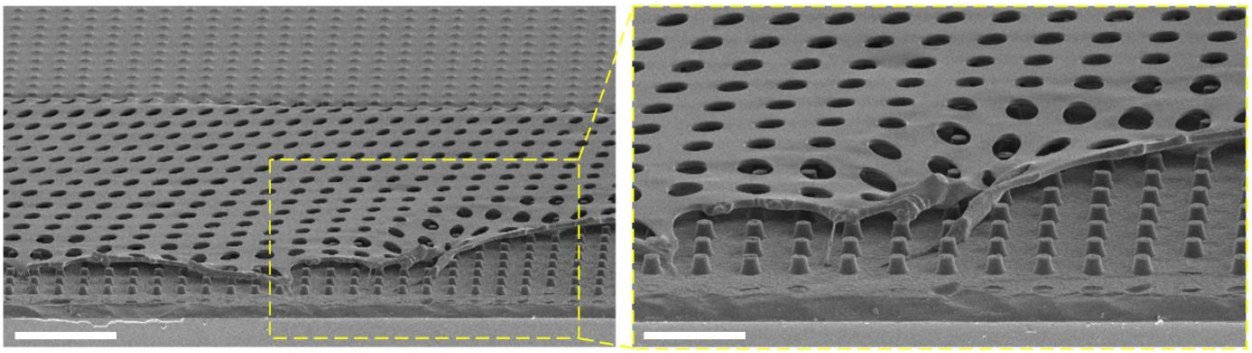

Fig. S3 Cross-sectional HR-SEM image (scale bar: 20 µm) of a PDMS-coated PR column array with the pitch of 5 µm, after etching for 30 s. The close-up HR-SEM image (scale bar: 10 µm) shows the peeling off and the stretching of the membrane (~960 nm thick) caused by breaking the sample, which resulted in larger through-holes.

Fig. S4 shows AFM images of the surface of a PDMS membrane etched at various etching times, and their corresponding top-view HR-SEM images. A slightly increase in the membrane surface roughness from 0.67 nm to 3.01 nm was obtained with an increase in the etching time from 15 s to 60 s, respectively. Such increase in the membrane surface roughness could also be observed in the top-view HR-SEM images.

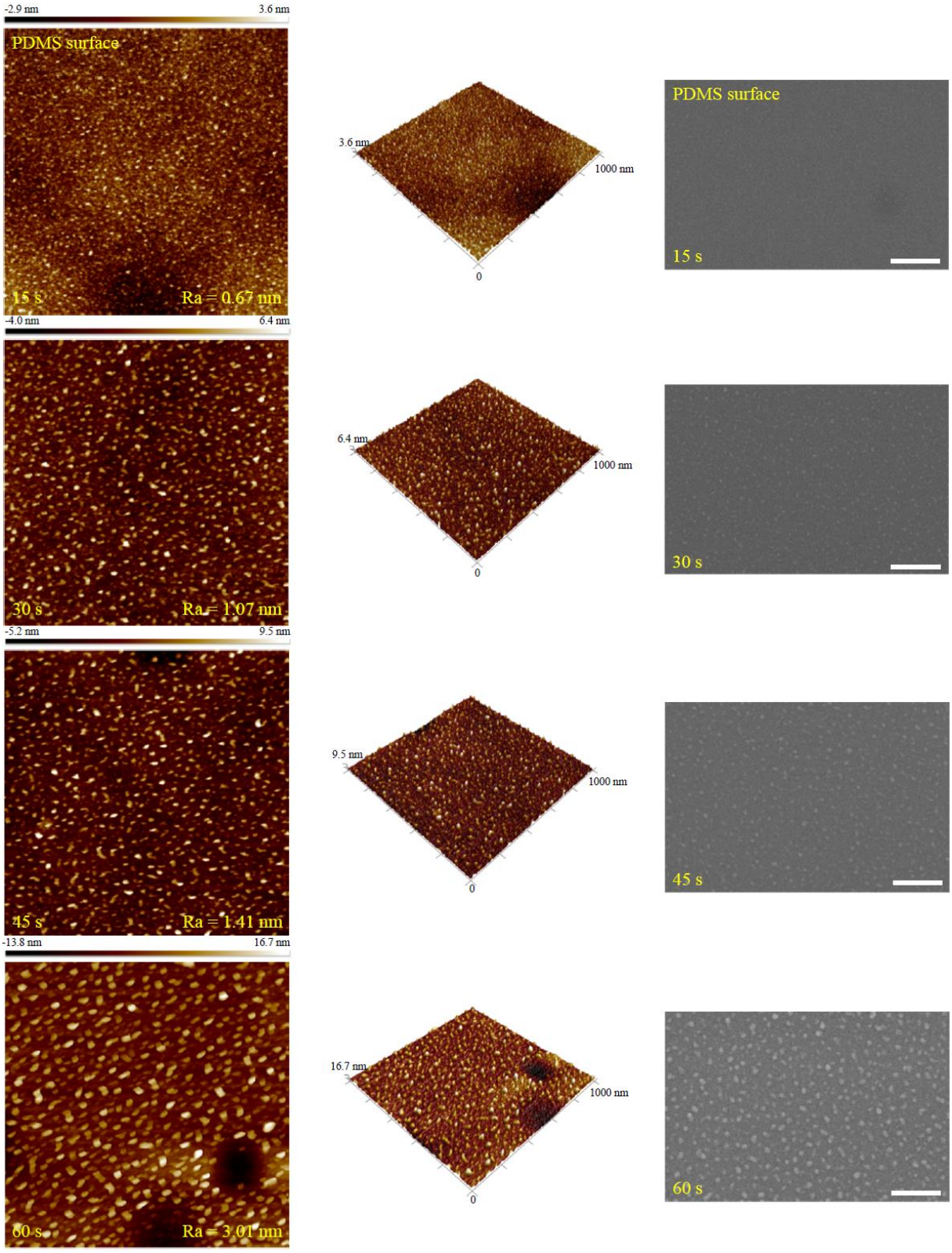

Fig. S4 AFM images (scan field: 1×1 μm^2) of the surface of a PDMS membrane etched at various etching times, and their corresponding top-view HR-SEM images (scale bar: 200 nm).

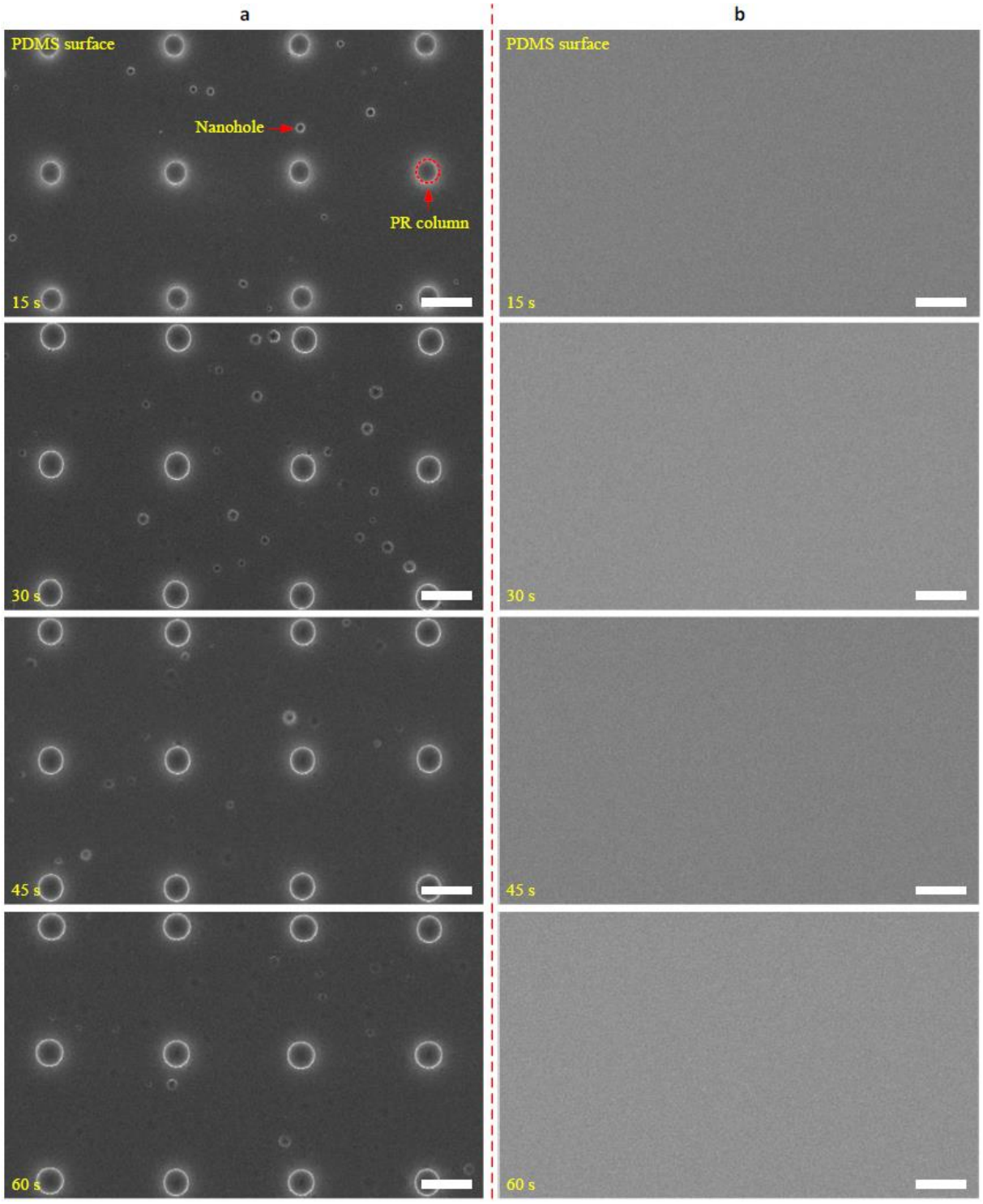

Fig. S5 Top-view HR-SEM images of the surface of PDMS membranes, etched at various etching times. The PDMS:hexane (1:10) solution was spin-coated over a) a PR column array (5 µm pitch), or on b) a sacrificial photoresist layer. Scale bars represent 2 µm.

Fig. S5 shows the top-view HR-SEM images of the surface of PDMS membranes etched at various etching times. After the etching process, nanoholes were only observed on the membrane surface coated over an PR column array (Fig. S5a). It is clearly seen that the number of these nanoholes slightly increased in the beginning (from 15 s to 30 s etching time), and subsequently decreased significantly with the increasing etching time. We attribute these nanoholes to the nanobubbles trapped inside the PDMS membranes. These nanobubbles were probably created during the spin-coating of this solution over the fabricated PR arrays at the high spinning speed of 6000 rpm. No nanohole observed on the membrane surface coated on a continuous photoresist layer indicated a sufficient degassing process of the PDMS:hexane solution (Fig. S5b).

Fig. S6 shows the energy dispersive X-ray spectrometry (EDS) spectrum and elemental quantitative data measured on a PDMS membrane surface after etching for 60 s. Sulfur (S) residues were found on the PDMS membrane surface, though at a relatively low percentage of approximately 1.5 wt%.

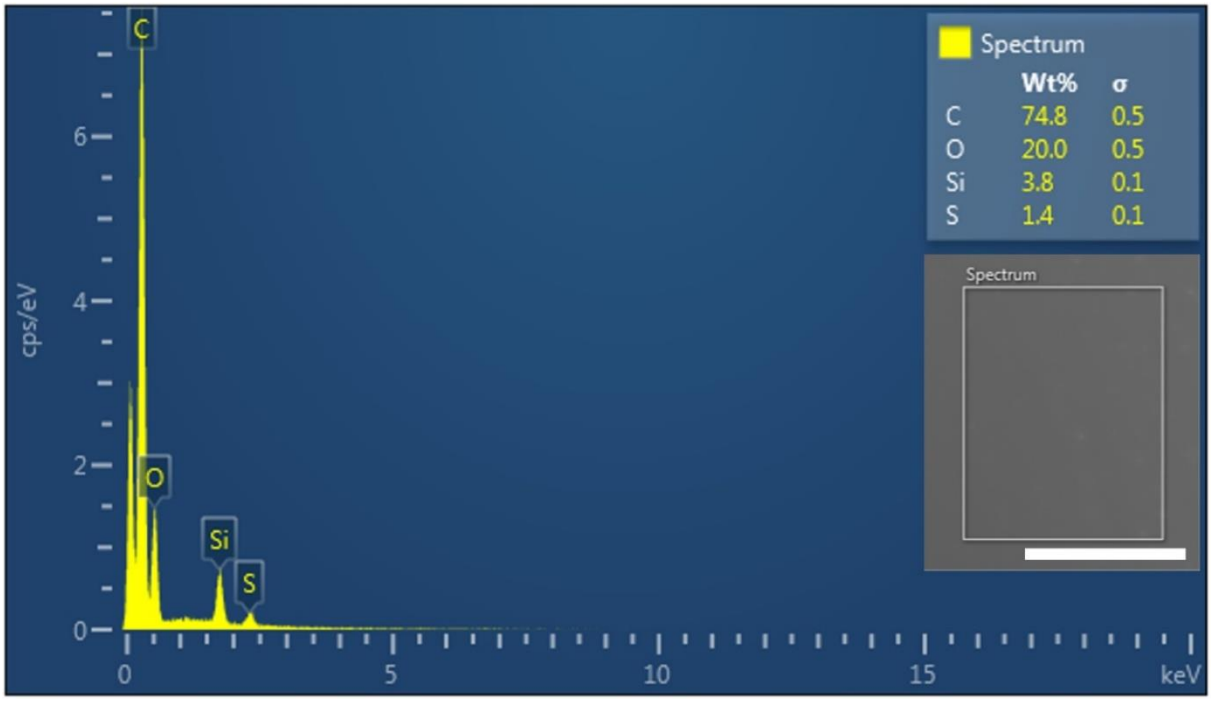

Fig. S6 EDS spectrum and elemental quantitative data measured on a PDMS membrane surface after etching for 60 s. The inserted HR-SEM image shows the measured area (scale bar: 500 µm).

SI4. Releasing of sub-µm PDMS through-hole membranes

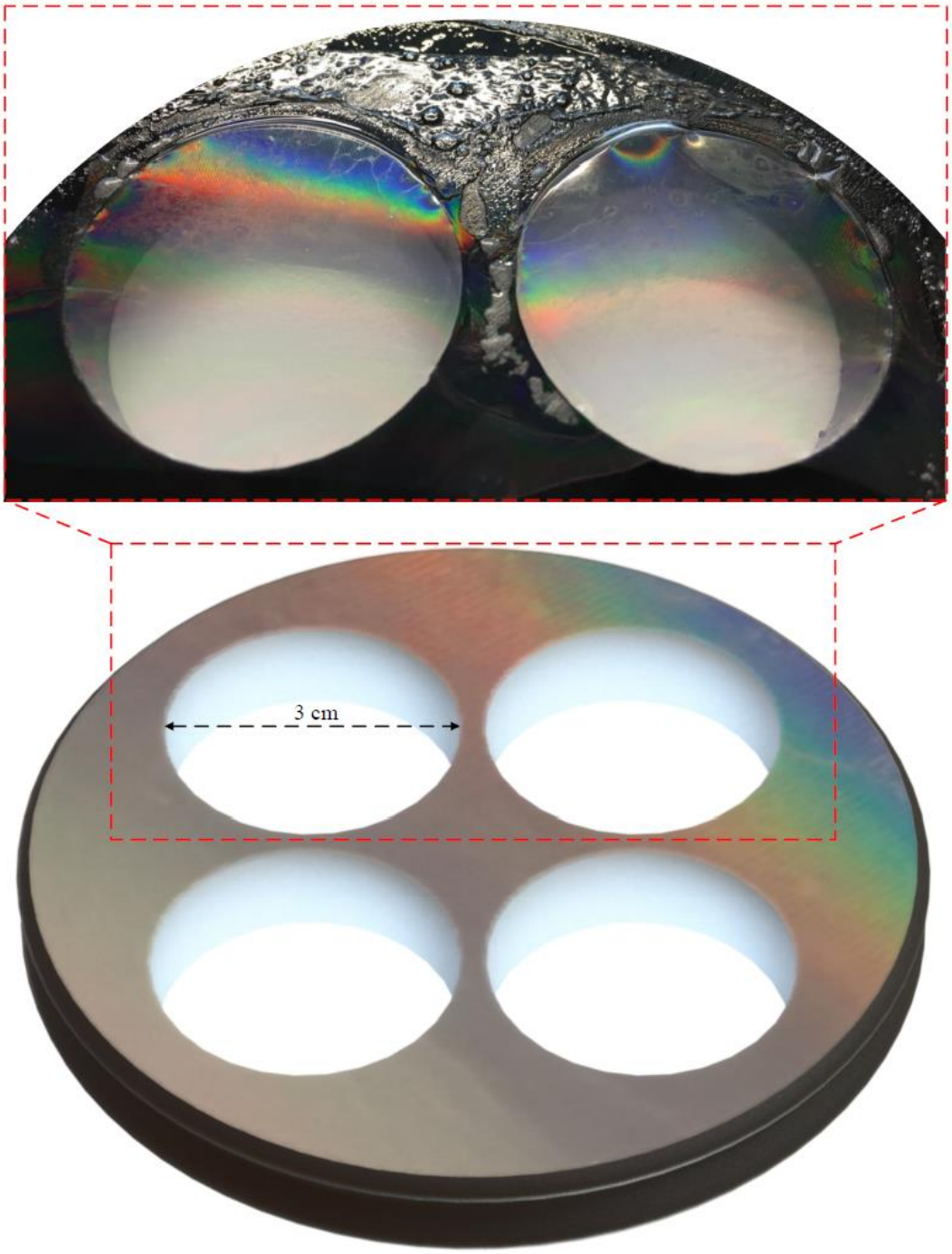

Fig. S7 Sketch of sub-µm PDMS through-hole membranes, which are free-standing on a 3D-printed ring used as a support. The inserted images show real optical images of the released PDMS membranes over large-areas of 3 cm in diameter.

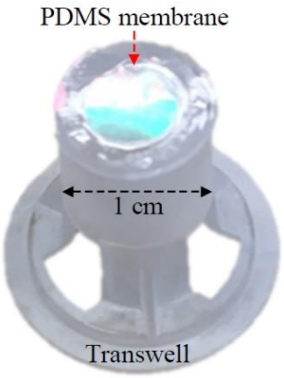

Fig. S8 Photograph of a sub-µm PDMS through-hole membrane, mounted on a support ring to create a Transwell-like culture set-up.

SI5. Cell culture on the fabricated PDMS membrane in a Transwell-like set-up

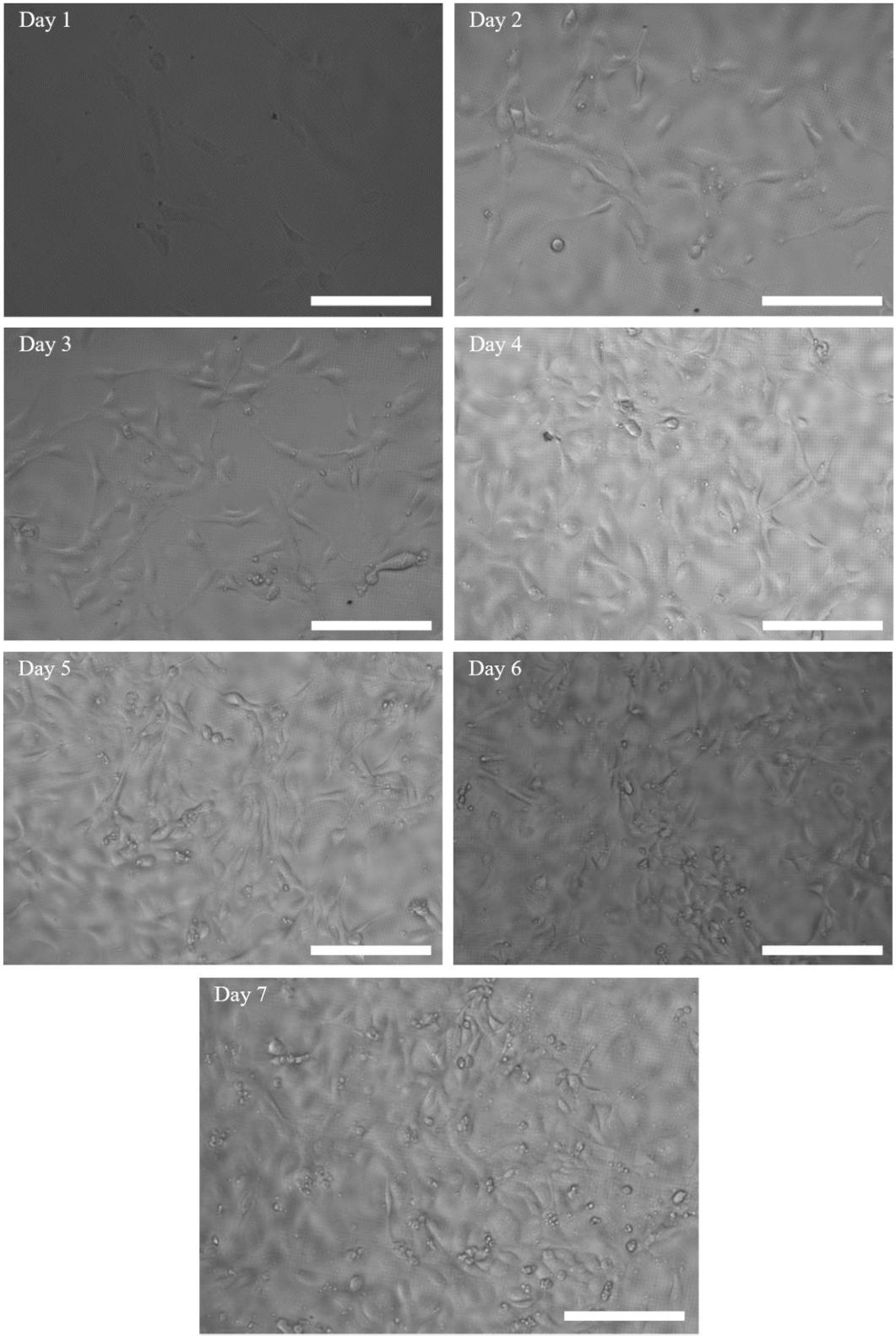

Fig. S9 Bright-field optical images of cultured cells on sub-µm PDMS through-hole membranes, captured at different days using an inverted microscope. Scale bar represents 200 µm.

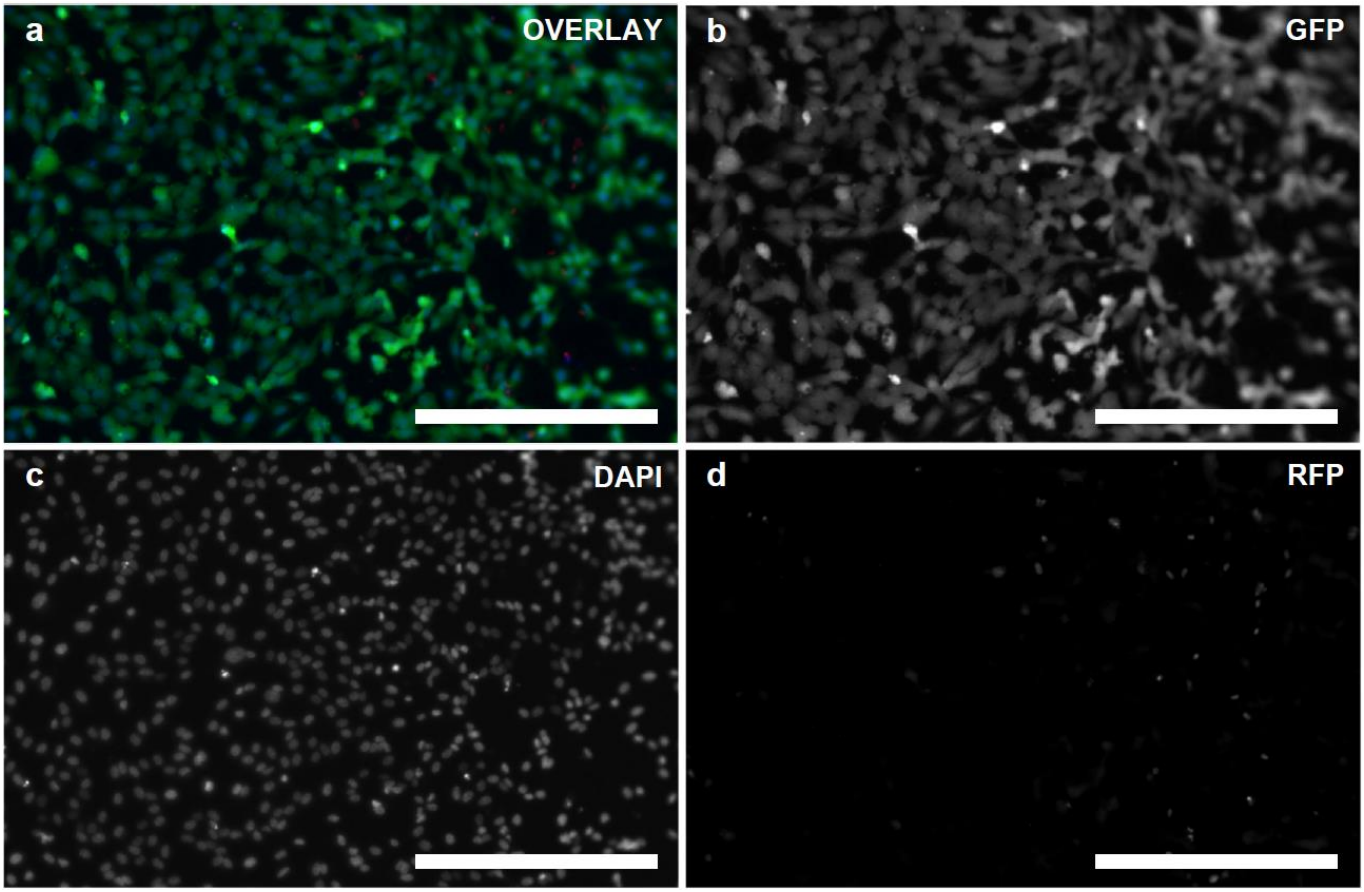

Fig. S10 Live/dead viability assay of HUVECs cultured on a PDMS membrane for 7 days. a) Overlay image of viable cells stained with Calcein AM (green), necrotic cells stained with Ethidium Homodimer (red) and Nuclei are stained with NucBlue™ Live ReadyProbes™ reagent (blue). b) Greyscale image of the Calcein AM staining, imaged by use of a Green Fluorescent Protein filter cube. c) Greyscale image of the nuclei staining NucBlue™ using a DAPI filter cube. d) Greyscale image of dead cells stained by Ethidium Homodimer and imaged using a Red Fluorescent Protein filter cube. Scale bars represent 400 µm.

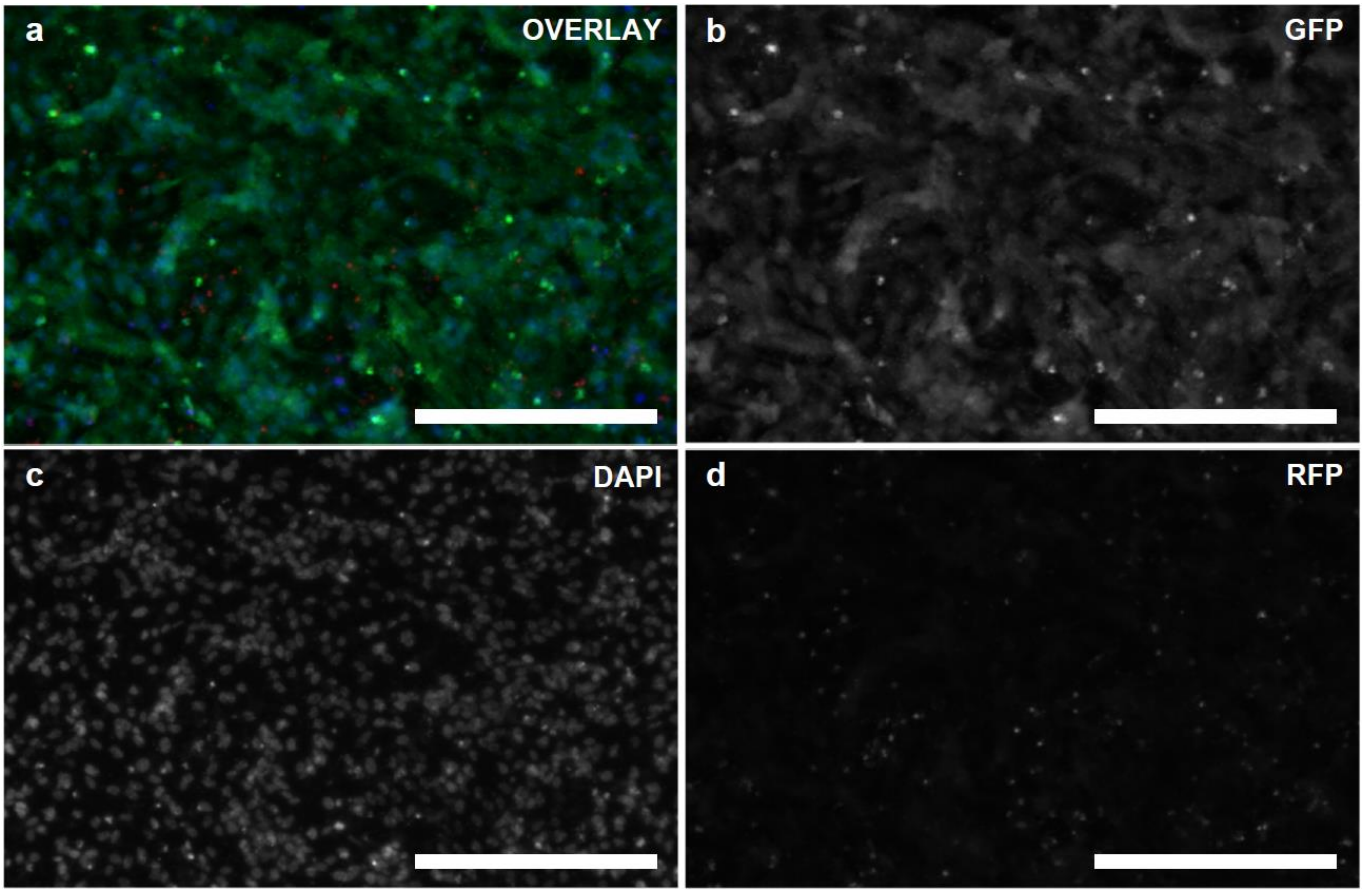

Fig. S11 Live/dead viability assay of HUVECs cultured on a Transwell permeable support (Corning Inc., USA) for 7 days. a) Overlay image of viable cells stained with Calcein AM (green), necrotic cells stained with Ethidium Homodimer (red) and Nuclei are stained with NucBlue™ Live ReadyProbes™ reagent (blue). b) Greyscale image of the Calcein AM staining, imaged by use of a Green Fluorescent Protein filter cube. c) Greyscale image of the nuclei staining NucBlue™ using a DAPI filter cube. d) Greyscale image of dead cells stained by Ethidium Homodimer and imaged using a Red Fluorescent Protein filter cube. Scale bars represent 400 µm.

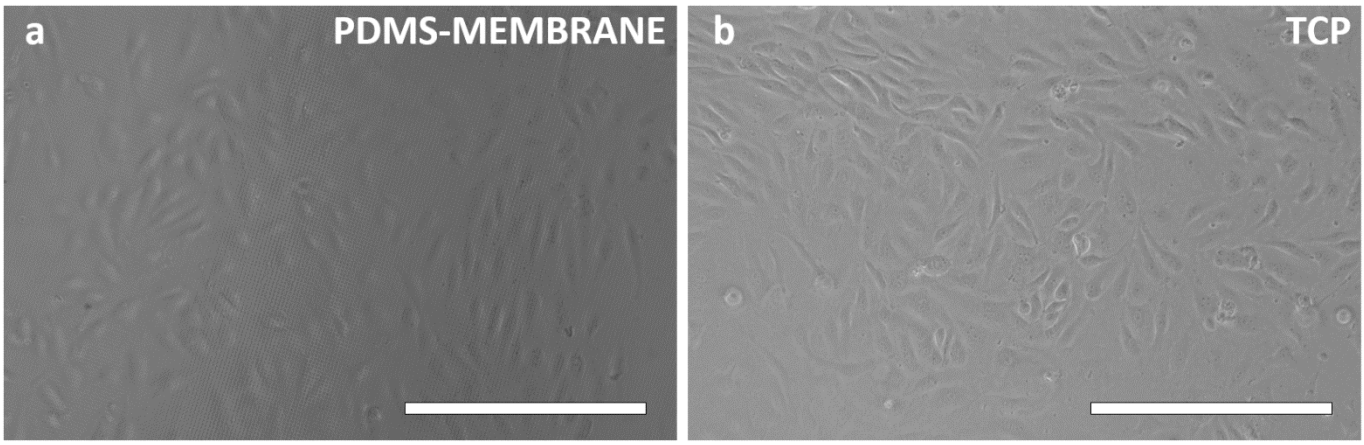

Fig. S12 Bright field images of HUVECs cultured on a) a sub-µm through hole PDMS membrane and b) a tissue culture plastic (TCP) substrate. No significant differences in cell morphology are observed. Images are taken 24 hours after cell seeding. Scale bars represent 400 µm.

SI6. Integration of sub-µm PDMS through-hole membranes into microfluidic chips

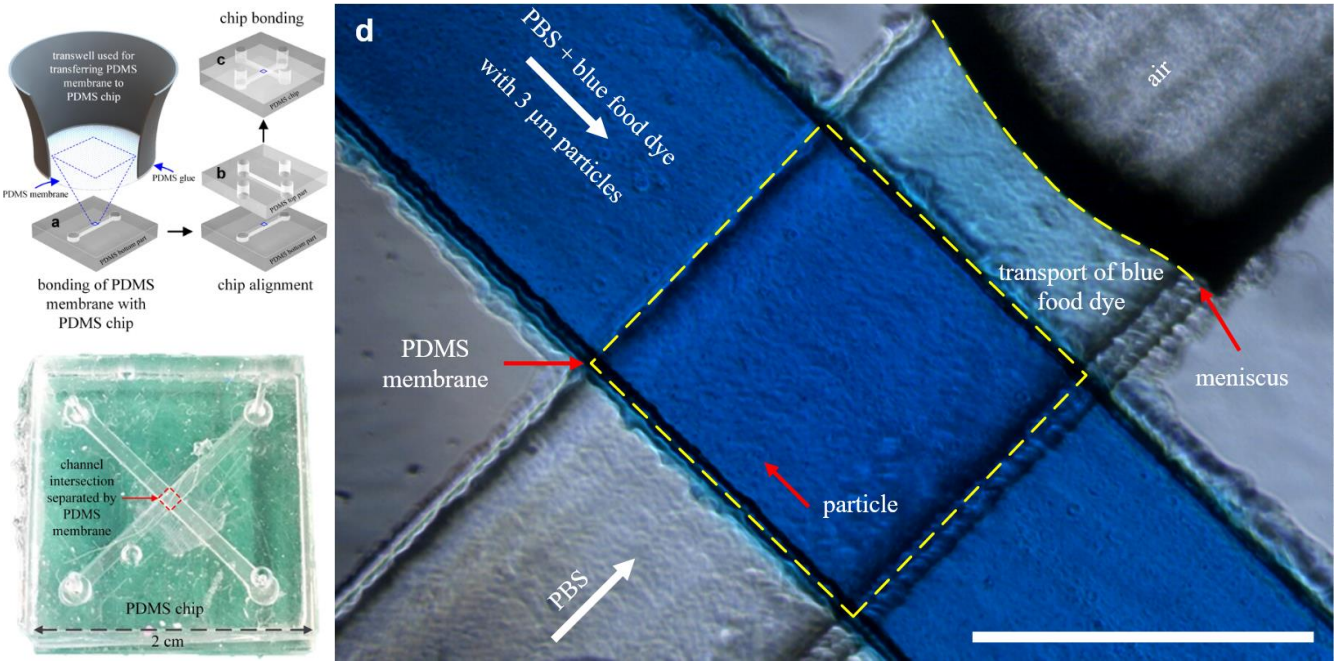

Fig. S13 a-c) Fabrication process for transferring a fabricated PDMS membrane to a PDMS microfluidic chip. d) A close-up optical image at the channel intersection separated by the PDMS through-hole membrane. Scale bar represents 800 µm.

Fig. S13a-c shows the fabrication process for transferring a fabricated PDMS membrane to a PDMS microfluidic chip using a supporting Transwell. Briefly, the bottom part of the PDMS chip was treated with O_2 plasma to activate its surface. Subsequently, a PDMS membrane mounted on a Transwell was brought into contact with the PDMS chip surface, thus resulting in a nice bond of the PDMS membrane with the bottom part surface of the PDMS chip. After releasing the Transwell, the bottom part of the PDMS chip with the PDMS membrane was aligned and bonded to the top part of the PDMS chip, which was already treated with O_2 plasma. The final PDMS chip was put in an oven at 60℃ for at least 1 h to achieve a good bonding. Fig. S13d shows the testing result of the fabricated PDMS chip. The mixture solution of phosphate buffered saline (PBS) and blue food dye with 3 µm diameter particles was flowed into the bottom channel of the PDMS chip at 1 µl min^{-1}. Whereas, only PBS solution was flowed into the top channel at the same speed of 1 µl min^{-1}. When the pure PBS solution flowed over the PDMS membrane, the blue food dye in the bottom channel went through the though-holes of the PDMS membrane to the top channel. This resulted in a mixture solution of PBS with blue food dye in the top channel. From the observation, the 3 µm particles were only flowed in the bottom channel without crossing the PDMS membrane with sub-micron through-holes.