SUPPLEMENTARY MATERIALS

Elastomeric microposts integrated into microfluidics for flow-mediated endothelial mechanotransduction analysis

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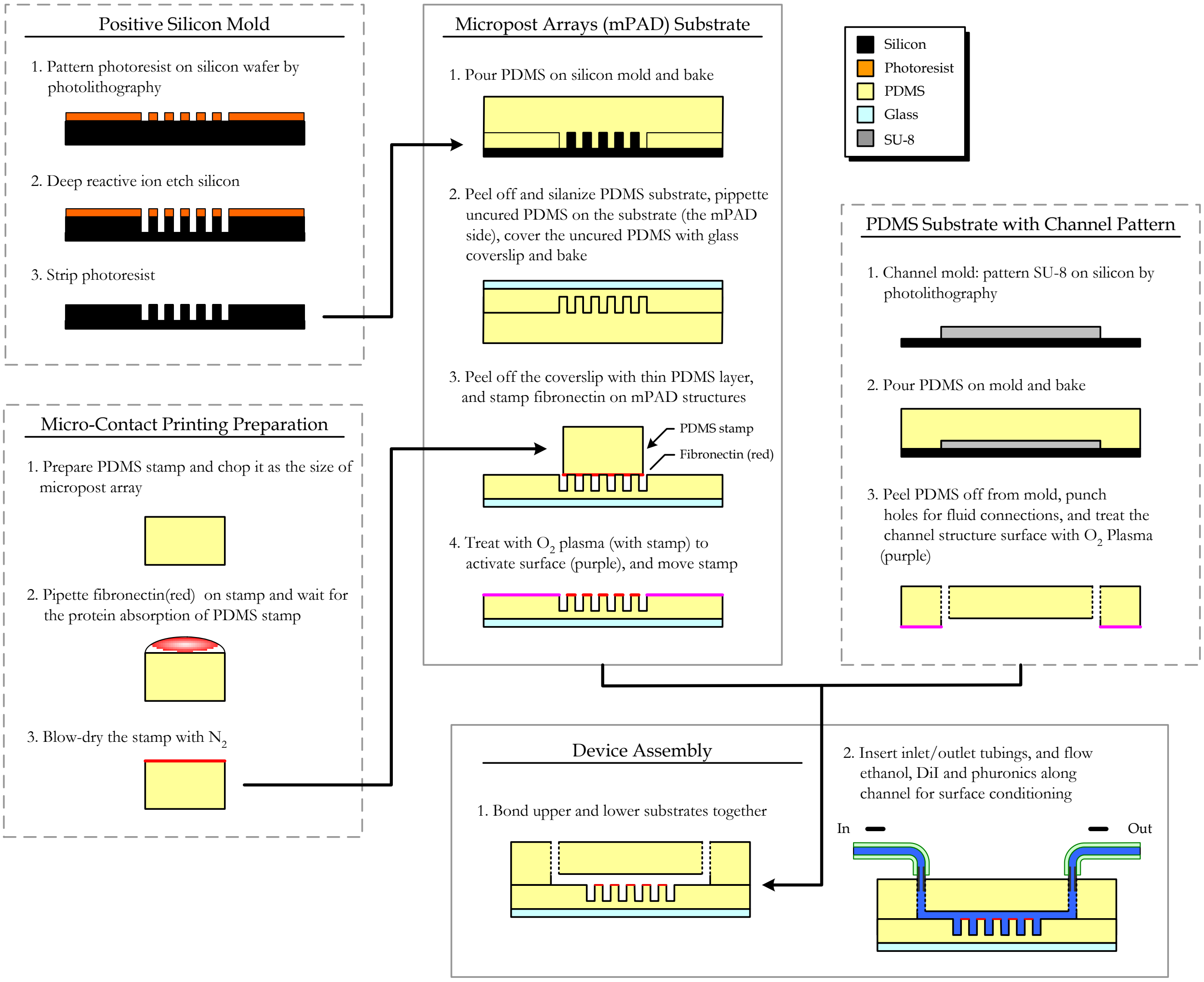
A. Supplemental Figures

Supplemental Figure S1



Positive Silicon Mold

1. Pattern photoresist on silicon wafer by photolithography
2. Deep reactive ion etch silicon
3. Strip photoresist

Micro-Contact Printing Preparation

1. Prepare PDMS stamp and chop it as the size of micropost array
2. Pipette fibronectin(red) on stamp and wait for the protein absorption of PDMS stamp
3. Blow-dry the stamp with N_2

Micropost Arrays (mPAD) Substrate

1. Pour PDMS on silicon mold and bake
2. Peel off and silanize PDMS substrate, pippette uncured PDMS on the substrate (the mPAD side), cover the uncured PDMS with glass coverslip and bake
3. Peel off the coverslip with thin PDMS layer, and stamp fibronectin on mPAD structures
4. Treat with O_2 plasma (with stamp) to activate surface (purple), and move stamp

PDMS Substrate with Channel Pattern

1. Channel mold: pattern SU-8 on silicon by photolithography
2. Pour PDMS on mold and bake
3. Peel PDMS off from mold, punch holes for fluid connections, and treat the channel structure surface with O_2 Plasma (purple)

Device Assembly

1. Bond upper and lower substrates together
2. Insert inlet/outlet tubings, and flow ethanol, DiI and phuronics along channel for surface conditioning

Fig. S1 Detailed fabrication process for the μPAC device. The detailed experimental procedures and parameters were discussed in **Methods**.

Supplemental Figure S2

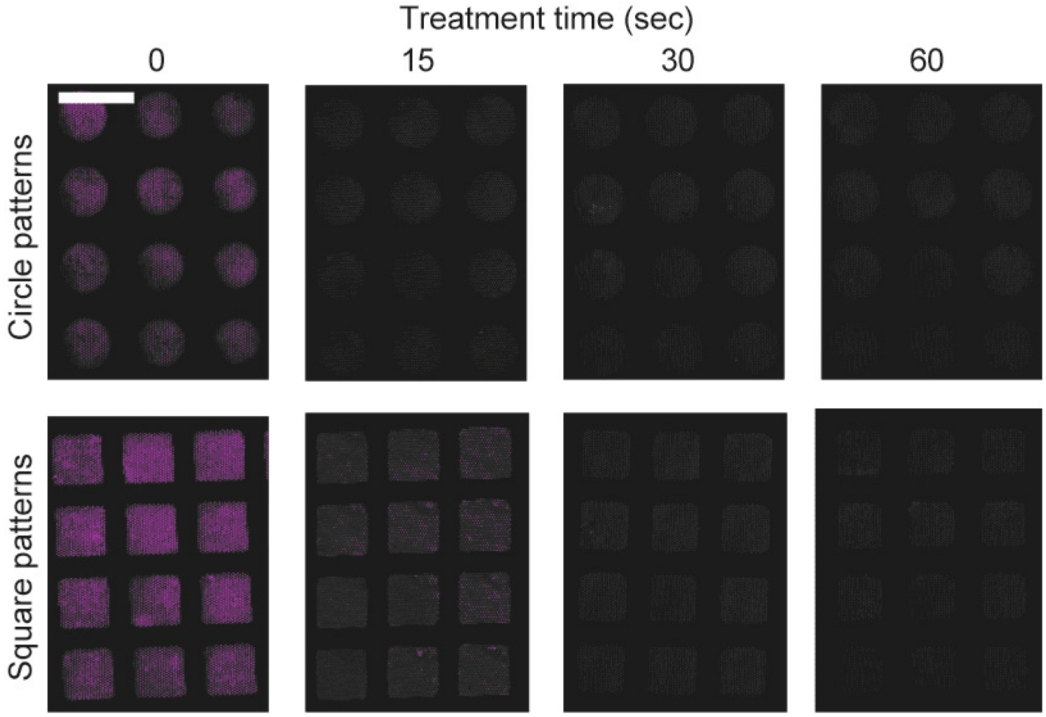


Fig. S2 Fluorescence images of BSA-coated PDMS microposts after treatments with the oxygen plasma with different durations, as indicated. Other treatment parameters were identical as described in **Methods**. Scale bar, 80 µm.

Supplemental Figure S3

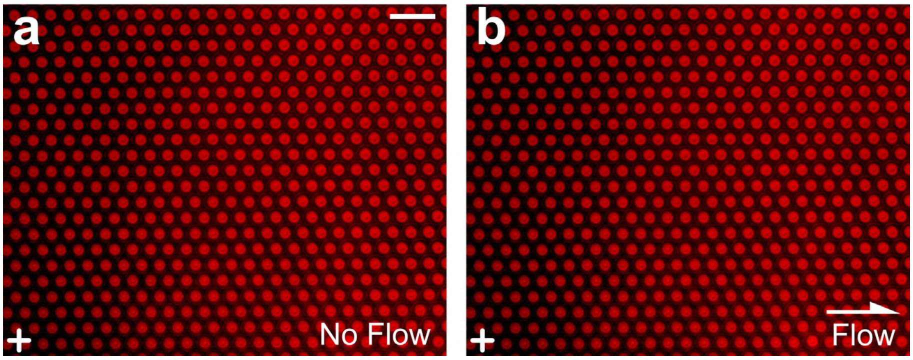


Fig. S3 Fluorescence images of micropost tops under (**a**) the static fluid condition and (**b**) a continuous flow with a shear stress of 20 dyne/cm^2. The deflection of the PDMS micropost under the shear stress was not measureable at all. The cross in the images marked an absolute reference location. Scale bar: 10 μm.

Supplemental Figure S4

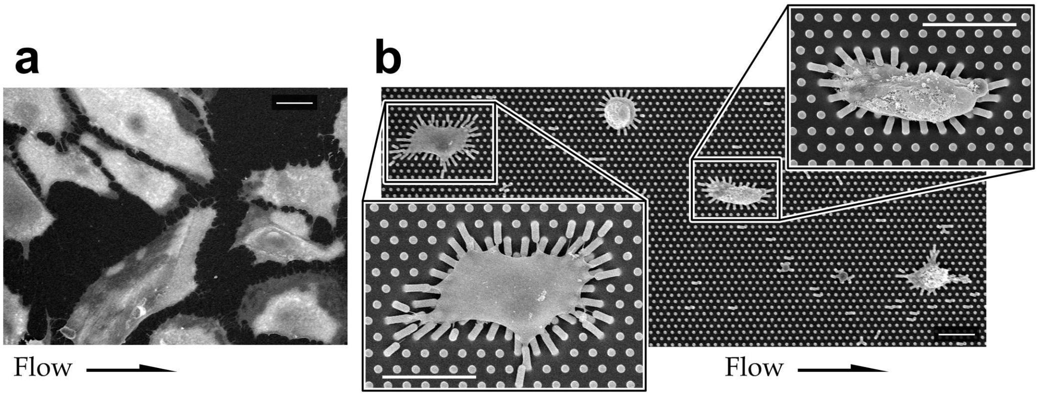


Fig. S4 SEM images of HUVECs that was aligned in the direction of the flow (**a**: HUVECs on the flat PDMS surface; **b**: HUVECs on the PDMS microposts). Scale bars, 20 μm.

Supplemental Figure S5

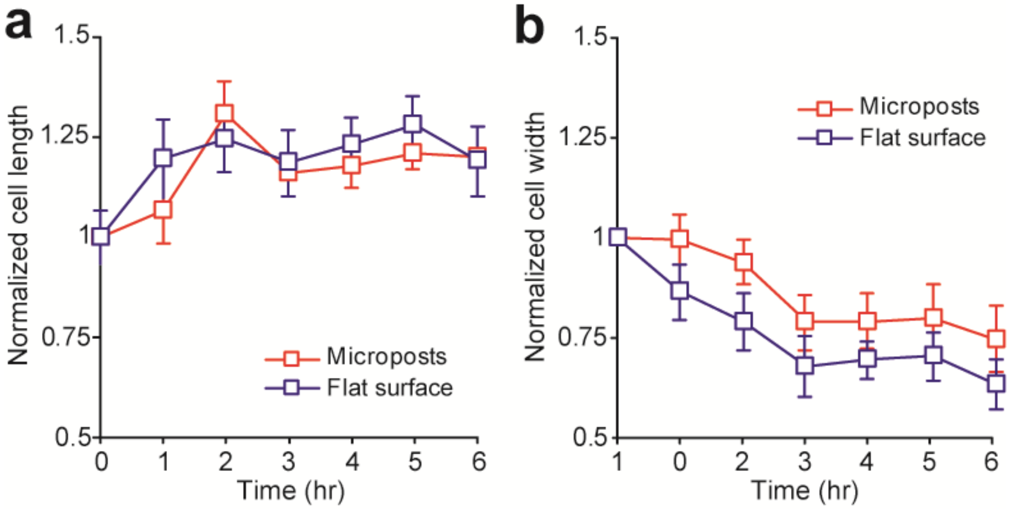


Fig. S5 Temporal response of the EC morphology during the shearing experiments in terms of the normalized cell length (**a**) and width (**b**) representing the cell dimensions along and perpendicular to the flow direction, respectively.

Supplemental Figure S6

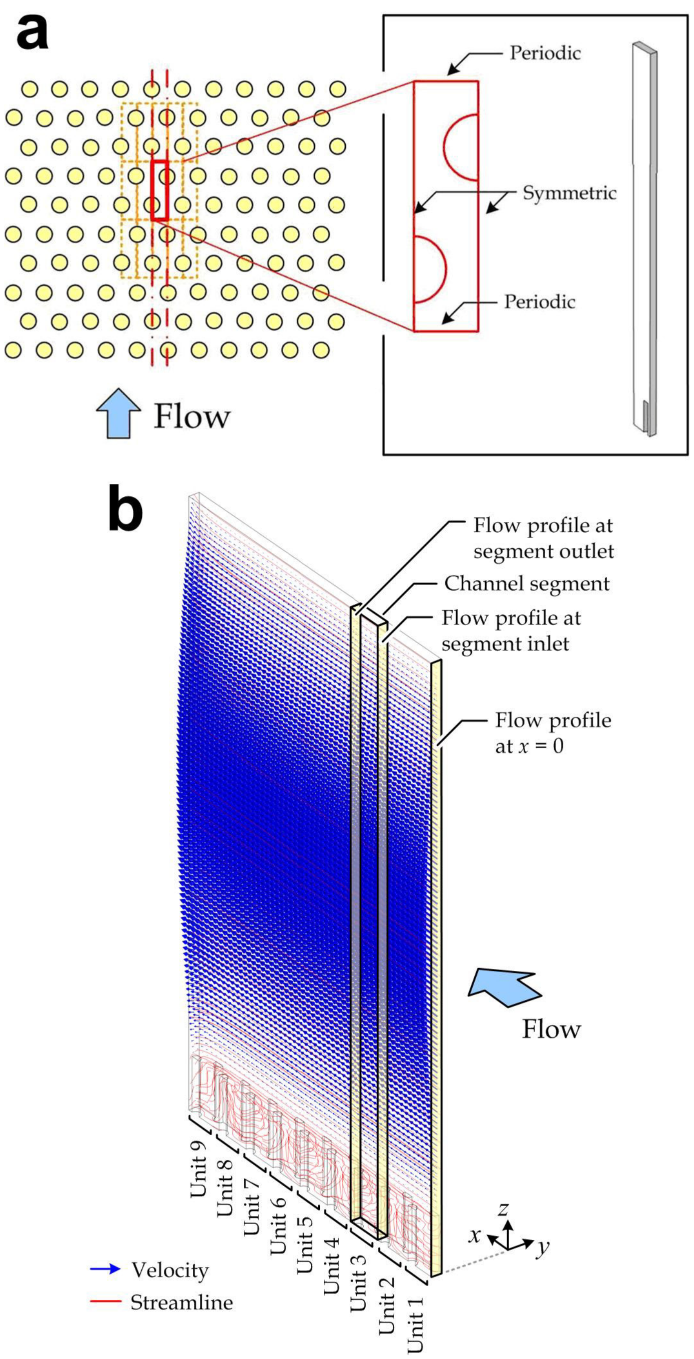


Fig. S6 (**a**) A repeating geometrical unit used for flow simulation to study the flow characteristics in the μPAC device. (**b**) Simulation model containing 9 repeating geometrical units in the flow simulation.

B. Simulation procedures for flow profile around microposts

Simulation of the flow properties in the μPAC device was performed using the commercial software (COMSOL 4.1) to estimate the shear stress generated on the tops of the PDMS microposts during the device operation. Here we selected a rectangular parallelepiped-shaped repeating element as the simulation unit (with the unit length of 6.93 μm, width of 4 μm, and height of 109 μm), which was composed of two halves of the PDMS microposts (**Fig. S6a**). The boundary conditions of this simulation unit were: (1) the no-slip boundary condition on all the PDMS surfaces (the tops and shafts of the PDMS microposts and the channel base and ceiling); (2) the symmetric boundary condition on its two side faces; (3) the periodic boundary condition for its entrance and exit faces.

The medium flow rate from the inlet of the μPAC device Q was set as 0.4 ml/min to establish the targeted shear stress of 20 dyne/cm^2. Based on simple geometrical arguments, the inlet flow rate Q_m of the simulation unit was set to 1.6×10^{-3} ml/min. In the computation, a simple model containing 9 repeating simulation units was constructed (**Fig. S6b**). The target simulation unit for calculating the shear stress on the top surface of the PDMS micropost was selected as the 'unit 3' in the model, while the other 6 downstream repeating simulation units (unit 4-9 in **Fig. S6b**) were added to capture the disturbing effect caused from the model outlet. The first two boundary conditions described above were achieved by adjusting settings in the COMSOL software, while the periodic boundary condition for the entrance and exit faces of the simulation unit was resolved by iterative simulation procedures described as follows:

1. A uniform velocity profile was first set at the entrance of the 9-unit model along the inlet plane at $x = 0$. The velocity profile over the whole 9-unit model was then computed.
2. Starting from the second iteration, the flow profile at the outlet of the 'unit 3' was extracted and further used as the flow profile at the entrance of the 9-unit model along the inlet plane at $x = 0$. The velocity profile over the whole 9-unit model was then computed.
3. The difference between the flow velocities at the entrance and exit faces of the 'unit 3' was calculated. If this difference would lie within a computation tolerance such that the difference of the absolute medium flow rates at the entrance and exit faces of

the 'unit 3' would be less than $10^{-5} \times Q_m$, we would proceed with step 4, otherwise we would repeat step 2.

4. The entire velocity profile in 'unit 3' was exported as the fully developed flow profile. Based on this velocity profile, we further calculated the shear stress at different horizontal planes along the height of the PDMS micropost (**Fig. 3c**).