Electronic Supplementary Information

High throughput and multiplex localization of proteins and cells for in situ micropatterning using pneumatic microfluidics

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Abstract. This supplementary information provides all the additional information of the current study.
Supplementary text

PµS control

To connect the control channel with Nitrogen gas for pressure supply, a control setup for the device operation was constructed. The control setup consisted of one set of eight-channel manifolds controlled through a NI-PCI-6513 controller board connected to a computer through a USB port. Nitrogen gas provided pressure (0-35 psi) to the manifolds. The control channels in the microfluidic device were first filled with water and were individually connected to the corresponding channels on the manifolds with metal pins using Tygon tubing, which also functions as the connector between the device and the manifold-involved controller for the supply of pressure during cell culture in the incubator. When a regulator on the manifold was activated, nitrogen gas entered the respective control tubing connected with the regulator, providing pressure to close the corresponding valves in the microfluidic device. The control interface was created using LabVIEW program (Version 8.0, National Instrument) on a computer, allowing for the manual control of individual valves.
Fig. S1 One typical photograph of the actual microfluidic devices used in the current study. The inlet and outlet (diameter: 1.0 mm) of the fluidic layer were connected to PTFE tubes (external diameter: 1.2 mm). The inlets (diameter: 0.35 mm) of control layer were connected to Tygon tubings (internal diameter: 0.42 mm) using stainless steel tubes (outer diameter: 0.5 mm).
Fig. S2 The microfluidic device with round-shape PµSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.

Fig. S3 The microfluidic device with strip-shape PµSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.
**Fig. S4** The microfluidic device with square-shape PµSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.

**Fig. S5** The microfluidic device with wavy-shape PµSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.
Fig. S6 The microfluidic device with oval-shape PμSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.

Fig. S7 The microfluidic device with Taiji-shape PμSs. (A) Schematic representation of the functional components in the device. (B) Design of the control layer. (C) Design of the fluidic layer.
Fig. S8 The fluorescent (A) and optical (B) pictures of PµSs with on/off switch by air pressure. The red circles were pointed to the PµSs-blocked regions.
**Fig. S9** Surface passivation of PDMS using Pluronic F127. (A) Schematic presentation of the PDMS modification by Pluronic F127. (B) The contact angle of native PDMS (top) and Pluronic F127-modified PDMS (bottom). (C) Quantitative analysis of PDMS wettability before and after the modification.
Fig. S10 The optical images of PµSs-assisted micropatterning of HUVEC-C cells (A) and HepG2 cells (B) with various PµS shapes including round, strip, square, oval, Taiji-shaped, and wavy.
**Fig. S11** The schematic description of multiple types of protein and cell micropatterning using the controlled PµSs.
Fig. S12 The optical images of co-micropatterning of HUVEC-C cells and HepG2 cells with various shapes by a serial manipulation using the well-designated PµSs for multi-step localization of different types of cells.