

Electronic Supplementary Information (ESI) for Lab on a Chip  
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## Electronic Supplementary Information

# Dynamic trapping and high-throughput patterning of cells using pneumatic microstructures in a microfluidic device

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**Abstract.** This supplementary information provides all the additional information and a more detailed discussion of the current study.

## ESI Movies

**Movie S1.** Cell trapping of the U-P $\mu$ S at the resting mode (0 psi pneumatic actuation and 10  $\mu$ L/min flow). During the resting mode, no cells were captured in the U-P $\mu$ S because of its two-dimensional structure.

**Movie S2.** Cell loss during the U-P $\mu$ S trapping (4 psi pneumatic actuation and 40  $\mu$ L/min flow). The trapped cells are easily removed in the initial-action mode of the U-P $\mu$ S at a fast flow rate.

**Movie S3.** Cell trapping of the U-P $\mu$ S at the initial-action mode (5 psi pneumatic actuation and 30  $\mu$ L/min flow). The cells are smoothly captured in the U-P $\mu$ S without leakage.

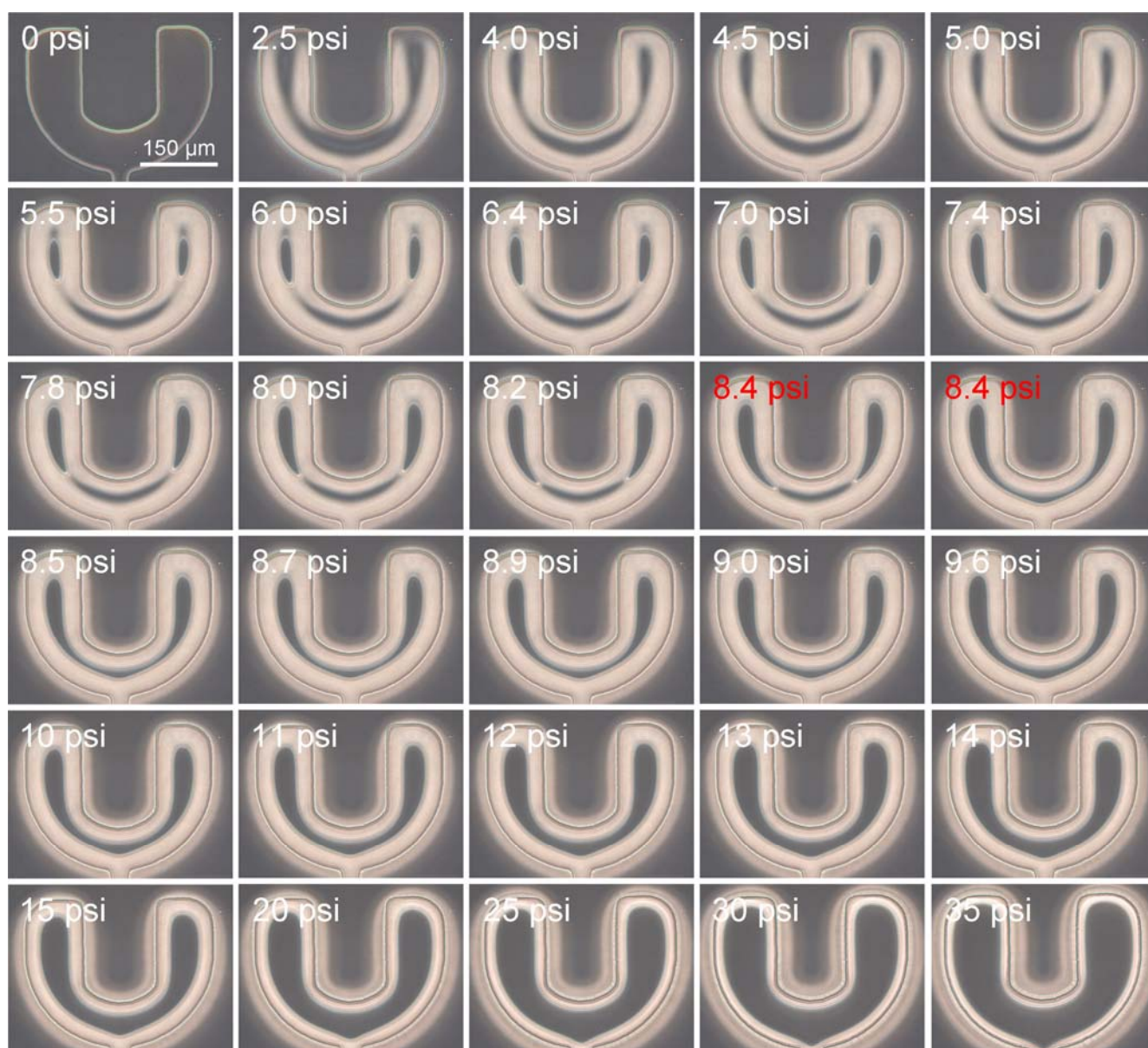
**Movie S4.** Hourglass-like cell loss during the U-P $\mu$ S trapping with the initial-action mode at high-speed flow (5 psi pneumatic actuation and 200  $\mu$ L/min flow). A higher flow rate can result in the leakage of the trapped cells, which is different from the cell loss observed at lower pneumatic actuation capture.

**Movie S5.** Path change of the flow in the full-action mode (15 psi pneumatic actuation and 120  $\mu$ L/min flow). The U-P $\mu$ S closes the road passage of the cell suspension, and that trapped cells are washed away at a high flow rate.

**Movie S6.** Cell trapping of the U-P $\mu$ S in the full-action mode (10 psi pneumatic actuation and 120  $\mu$ L/min flow). The back pressure at a high flow rate can allow flow passage to a certain extent through the U-P $\mu$ S with lower pneumatic actuation in the full-action mode. The capture of several cells is also shown in this movie.

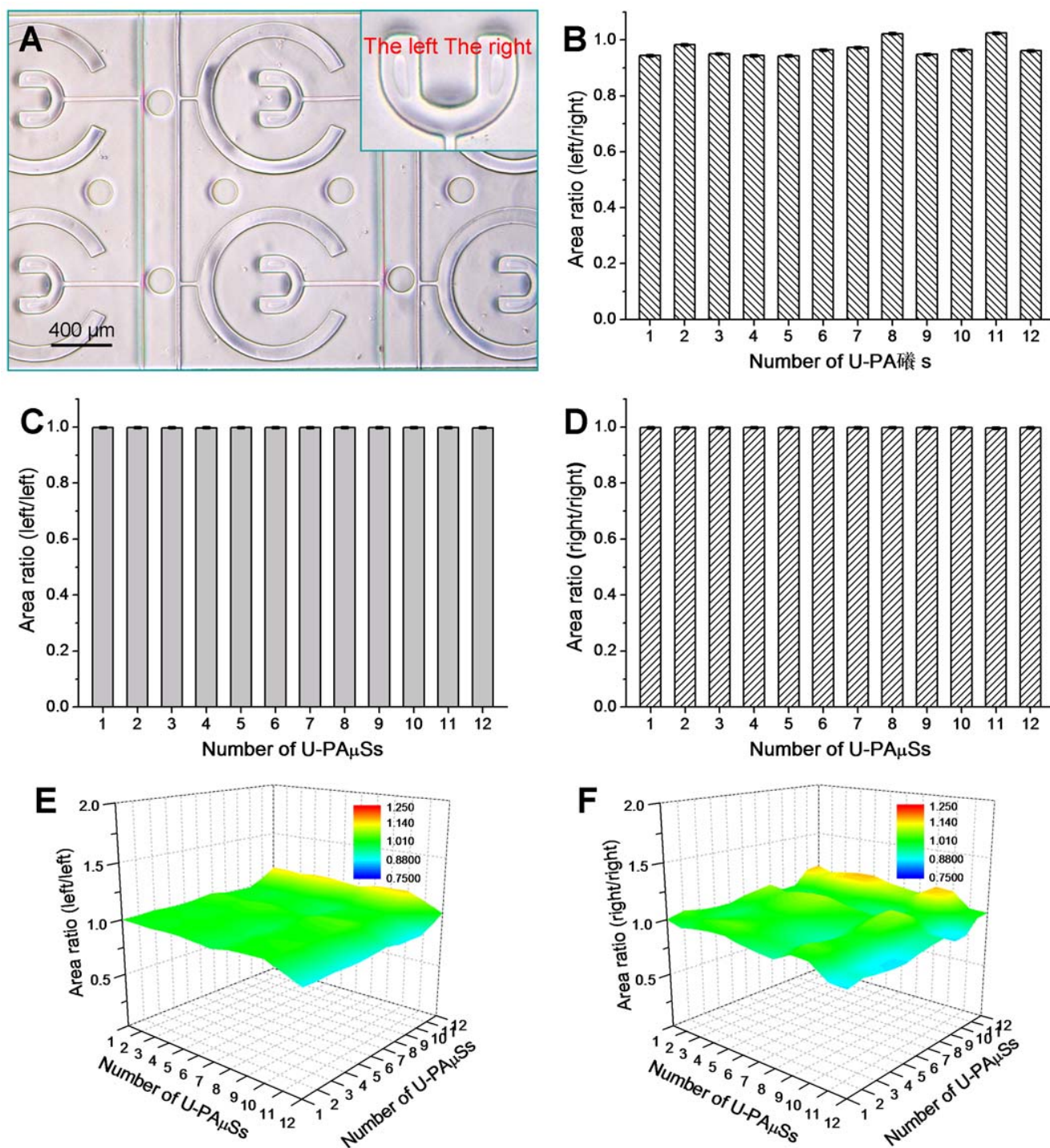
**Movie S7.** Mode conversion of the U-P $\mu$ S for the withdrawal of the trapped cells. This movie presents a cleaning process of the trapped cells from the U-P $\mu$ S by rinsing (120  $\mu$ L/min) and a

mode conversion of the U-P $\mu$ S from the initial-action mode to the resting mode (pneumatic actuation from 5 to 0 psi). The U-P $\mu$ S can realize a dynamic and precise positioning of cells.



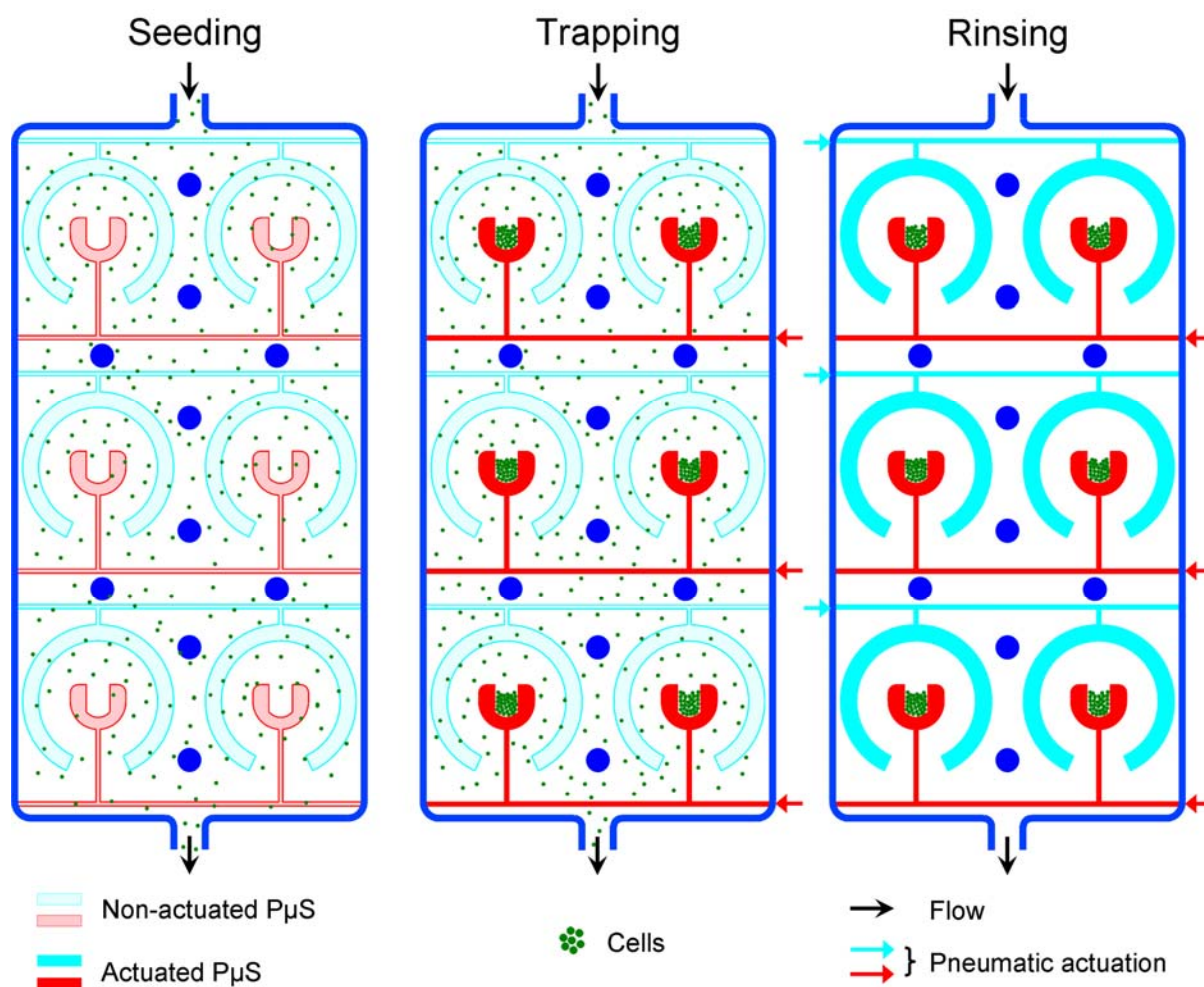
**Fig. S1.** Typical variable changes exhibited by the U-P $\mu$ S under different air pressures (0 psi to 35 psi) and zero flow. First, a two-dimensional U-P $\mu$ S was found at 0 psi pneumatic actuation. Second, a three-dimensional U-P $\mu$ S appeared as the air pressure increased. The PDMS membrane was swollen with the sequential and observable displays from slight uplift with non-touching of the ceiling of the chamber (2.5, 4.0, 4.5, and 5.0 psi), to part uplift with both sides touching the ceiling of the chamber (5.5, 6.0, 6.4, 7.0, 7.4, 7.8, 8.0, 8.2, and 8.4 psi), as well as to entirely uplift with U-shaped array touching the ceiling of the chamber (8.4, 8.5, 8.7, 8.9, 9.0, 9.6, 10, 11, 12, 13, 14, 15, 20, 25, 30, and 35 psi). Red-labelled images

present the qualitative U-P $\mu$ S shape alteration from both sides touching the ceiling of the chamber to the U-shaped array touching at 8.4 psi pneumatic actuation. These pictures demonstrate that a higher pneumatic actuation can lead to a higher-grade membrane deformation, and the nonuniform design of the U-P $\mu$ S results in its various shape exhibitions along with the different pneumatic actuations. The results above were determined based on the refractive appearance of the U-P $\mu$ S as shown in the optical microscope system (Olympus, CKX41). The method was repeated thrice using six batches of the fabricated devices. The pneumatic range of different U-P $\mu$ S states was described as mean  $\pm$  S.D. psi in the current paper.



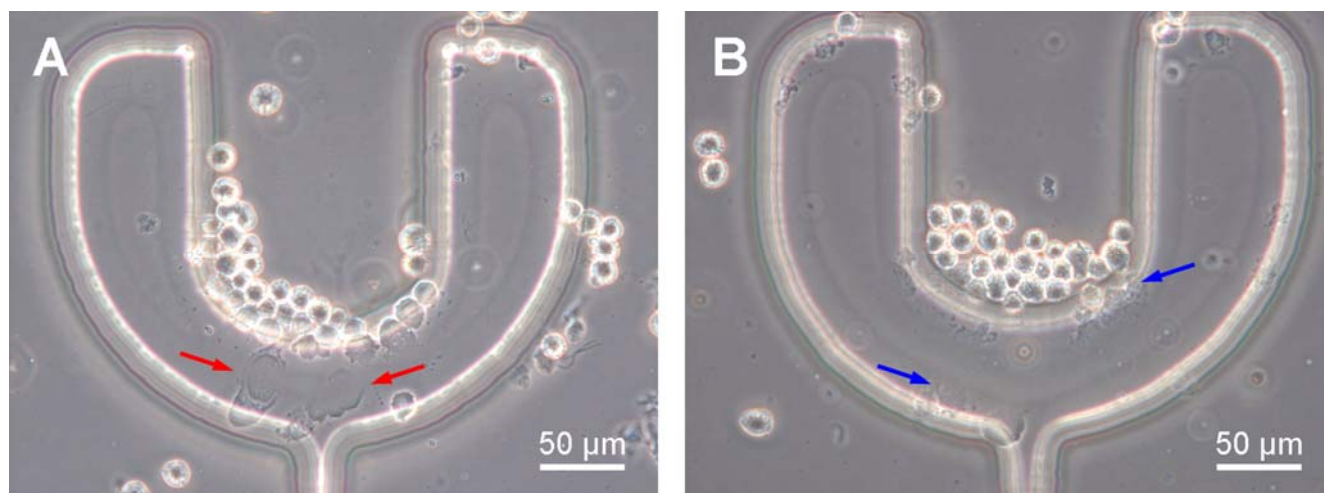
**Fig. S2.** Spatial consistency assessment of the U-PμSs in the microfluidic device. (A) Optical image of the actual U-PμSs (7.3 psi pneumatic actuation and 0 μL/min flow). Inset shows the enlarged picture of the U-PμS. (B) Area ratios of the left and right sides. The areas point to the touching areas of the U-PμSs to the ceiling of the microchamber. In this pneumatic actuation, both sides of the microstructures were in contact with the ceiling (defined here as the left and

the right, which correspond to the inset of A). Their touching areas were determined to investigate the three-dimensional structure consistency of the U-P $\mu$ Ss because it is convenient for quantitative calculation. (C) Area ratios of each of the two lefts of the U-P $\mu$ S between two pneumatic actuations. (D) Area ratios of each of the two rights of the U-P $\mu$ S between two pneumatic actuations. (E) Area ratios of the lefts between each of the two U-P $\mu$ Ss. (F) Area ratios of the rights between each of the two U-P $\mu$ Ss. The results show that the spatial structure of the U-P $\mu$ Ss is consistent and well controlled. This condition suggests that the U-P $\mu$ Ss are potentially fit to complete high-throughput cell trapping in a quantitative uniform manner. Twelve U-P $\mu$ Ss were applied for the current tests including the six U-P $\mu$ Ss showed in this figure A. All the data were from at least three repeated tests.

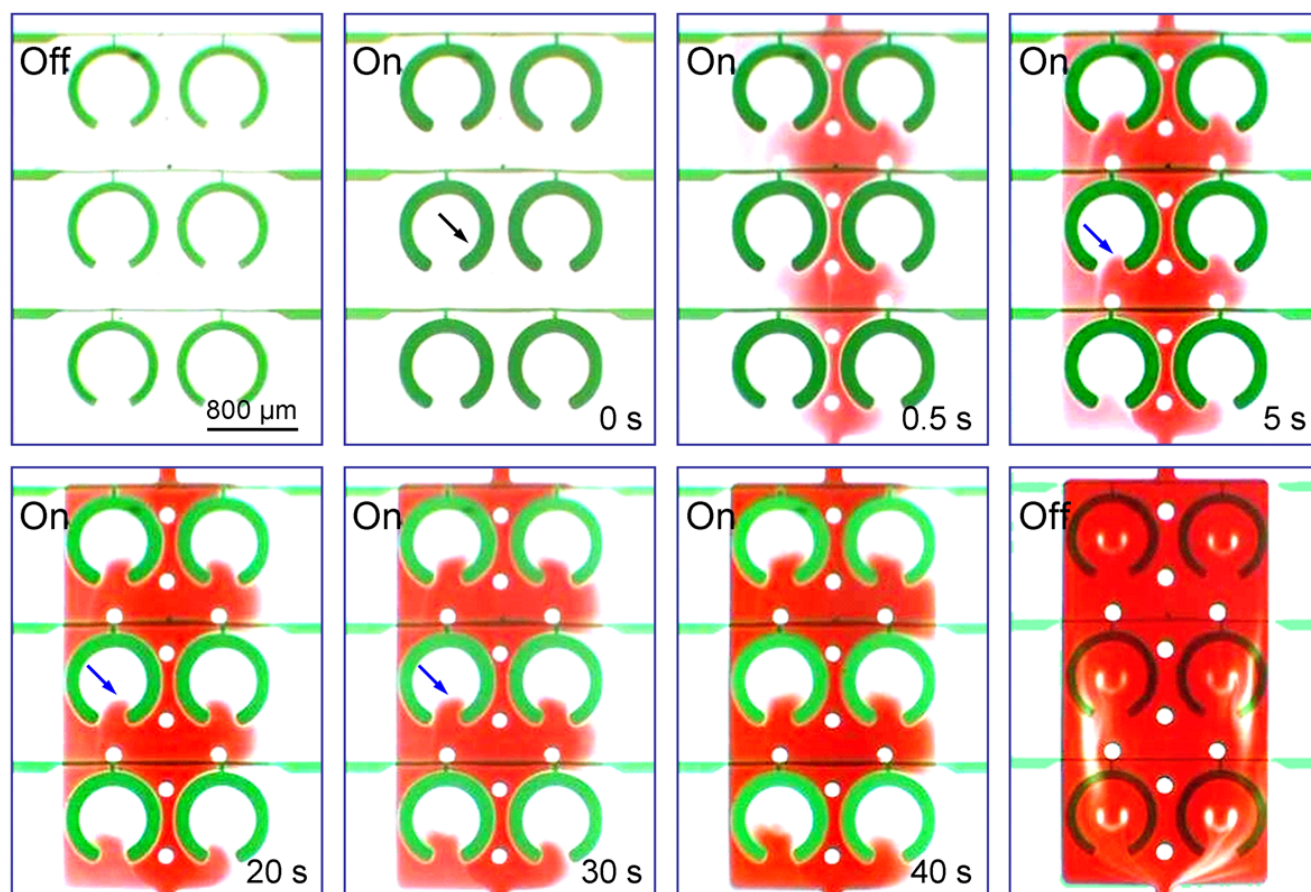


**Fig. S3.** Schematic diagrams of the cell trapping process using U-PμSs. During trapping, U-PμSs were actuated (red) from the resting state (light red). In addition, the umbrella-like PμSs were actuated (cyan) for a special protection of the trapped cells during the rinsing step.

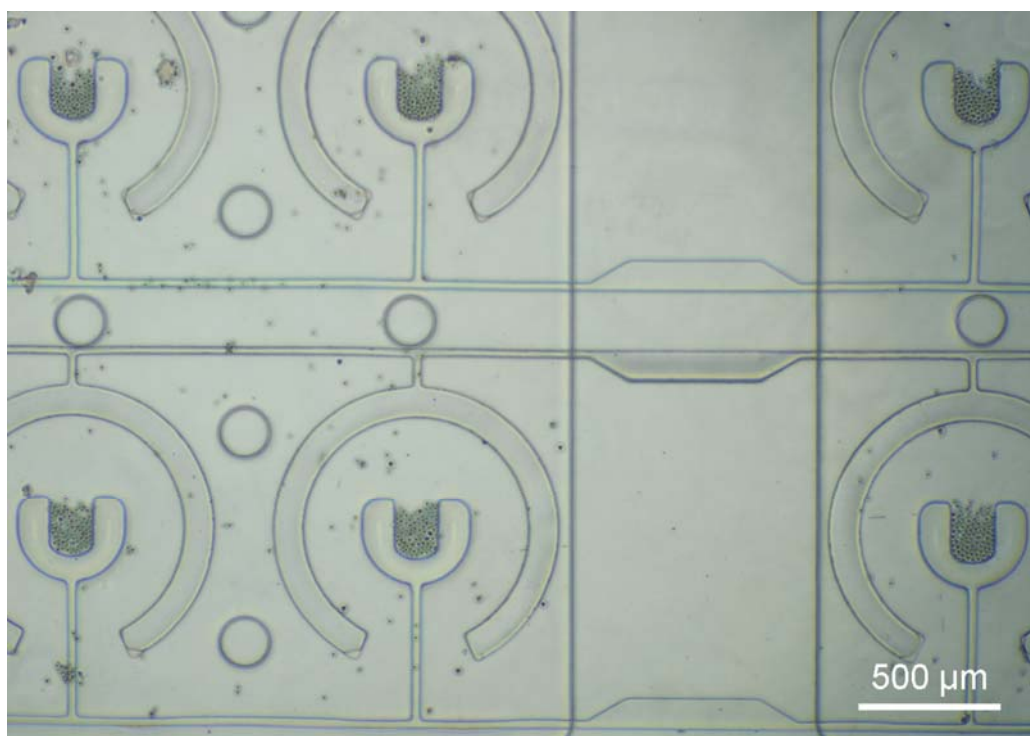




**Fig. S4.** Cell deformation during U-P $\mu$ S trapping at a high flow rate and high pneumatic actuation. (A) Cell trapping at 12 psi pneumatic actuation and 250  $\mu$ L/min flow. Several cells were obviously deformed (red arrows) in the gap between the U-P $\mu$ S and the ceiling of the microchamber. (B) Cell trapping at 15 psi pneumatic actuation and 400  $\mu$ L/min flow. Blue arrows show the cell debris. These results suggest that high flow rates and high pneumatic actuations could potentially result in the serious injury and direct necrosis of several cells. These conditions are not suitable for further applications involving live cell research, especially on the studies of cell function and behavior.



**Fig. S5.** Hydrodynamic blocking of the flow using the on/off switch of the umbrella-like P $\mu$ Ss. During pneumatic actuation (on, 30 psi), the umbrella-like P $\mu$ Ss expanded (black arrow) and served as physical barriers, and resulted in a detour of flow (red, 120  $\mu$ L/min). That is, the fluid cannot positively enter into the U-P $\mu$ Ss. Time lapse images show that the communication between the environments of the inner U-P $\mu$ Ss and flow mainly depends on molecular diffusion (blue arrows), implying that a nearly zero flow condition exists around the U-P $\mu$ Ss. Therefore, the actuated umbrella-like P $\mu$ Ss can prevent the negative effects of the flow on the trapped cells. In addition, during the cancellation of actuation (off, 0 psi) of the umbrella-like P $\mu$ Ss, the static flow condition around U-P $\mu$ Ss is replaced immediately by the perfusion flow. These results demonstrate that the on-demand protection of the trapped cells could be simply and conveniently realized via a real-time actuation of the umbrella-like P $\mu$ Ss.



**Fig. S6.** Parallel trapping of human hepatocellular liver carcinoma HepG2 cells using the U-P $\mu$ S arrays at 6 psi pneumatic actuation and 30  $\mu$ L/min flow.