Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supplementary Information

# Continuous Removal of Small Nonviable Suspended Mammalian Cells and Debris from Bioreactors Using Inertial Microfluidics

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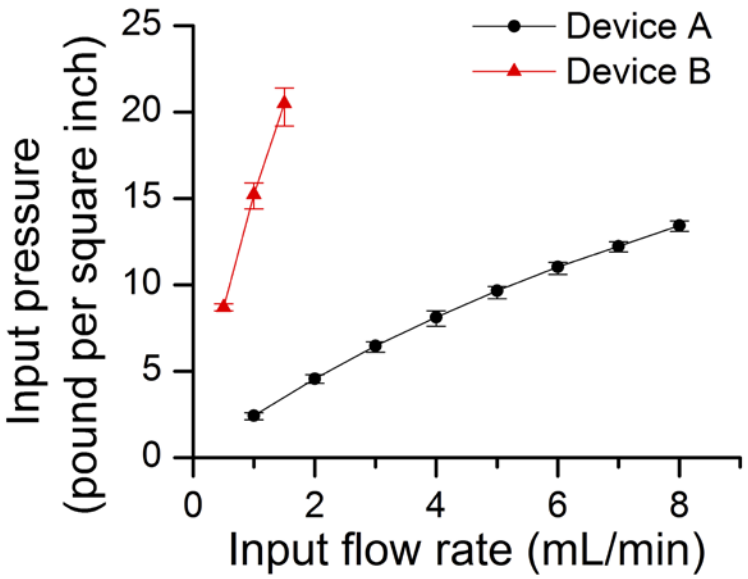
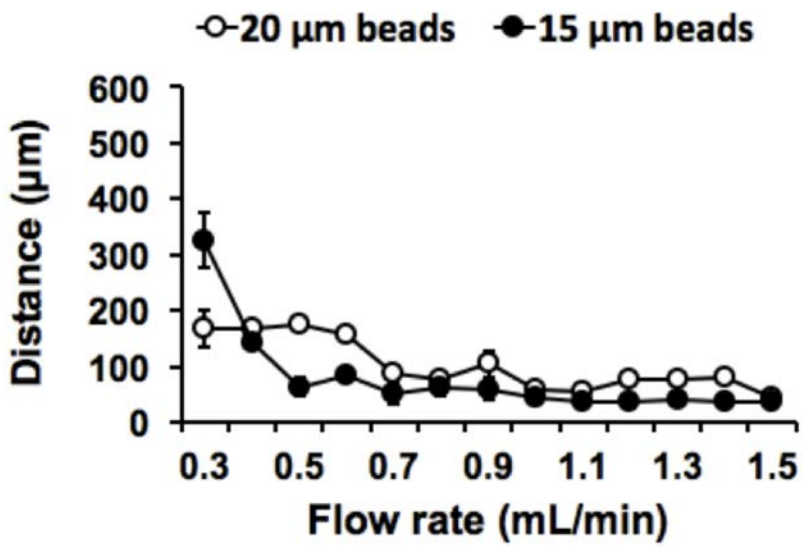
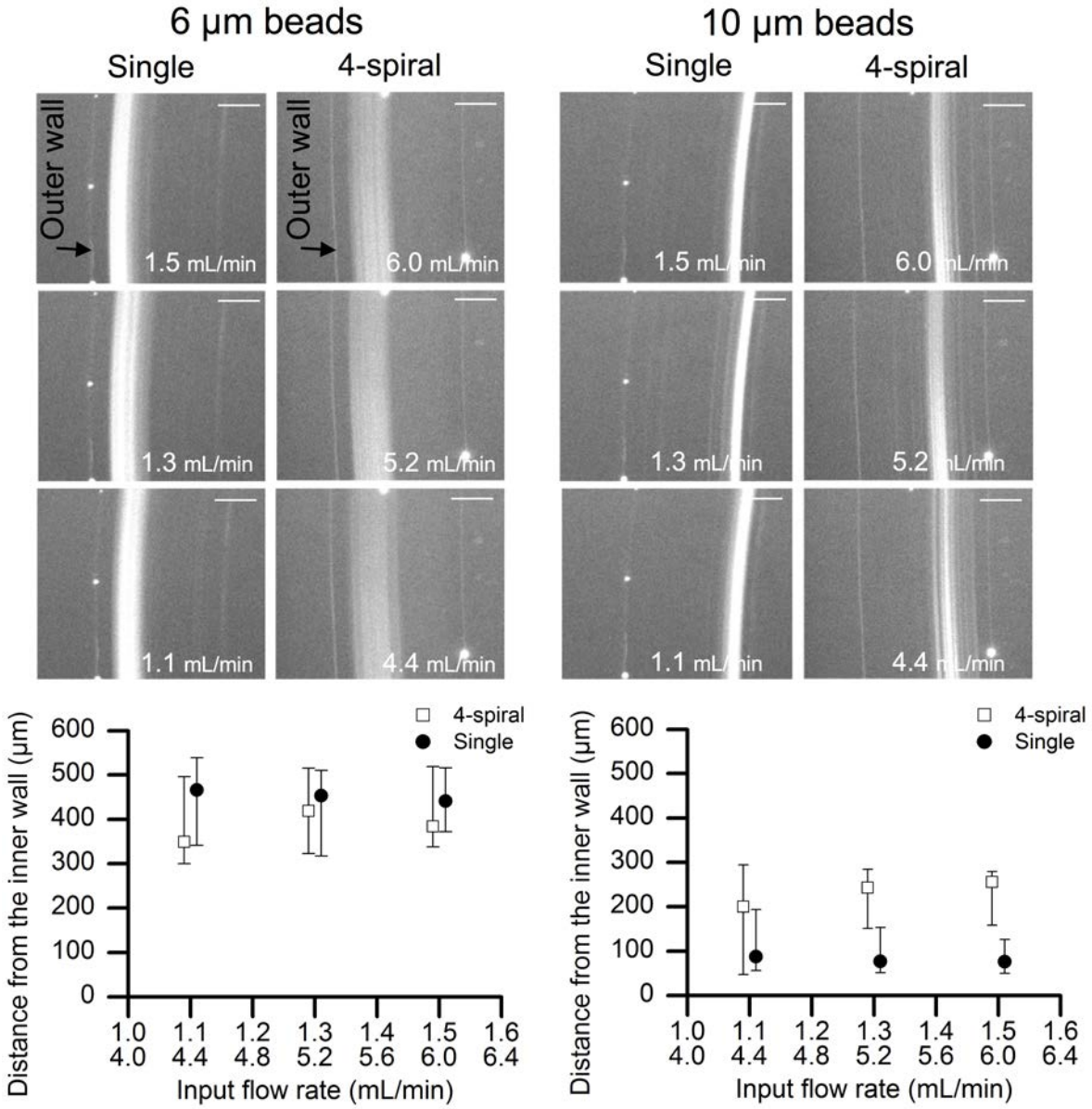



**Supplementary Figure 1** Comparison of input pressure between two devices (Device A: 200 µm inner depth, 140 µm outer depth, 1000 µm width, Device B: 80 µm inner depth, 130 µm outer depth, 600 µm width). Long-term (>days) continuous operation under input pressure of >20 pounds per square inch can lift off the PDMS piece from the bottom glass surface. Pressure was measured with a pressure flow cell (080-699PSX, SciLog, USA). Error bars, data range (*n* = 3, three different devices).




**Supplementary Figure 2** Focusing positions of 20 and 15 µm beads in the spiral channel (80 µm inner depth, 130 µm outer depth, 600 µm width). The positions are in direction of channel width. The distance 0 and 600 um correspond to the inner and outer wall of the channel, respectively. Error bars are the standard deviations of the bead positions.




**Supplementary Figure 3** Focusing behavior of fluorescent 6 and 10 μm beads (Fluoresbrite™ Plain YG Microspheres, Polysciences, USA) at the 6th loop in the single and 4-spiral devices. Input flow rates were varied from 1.5 mL/min to 1.1 mL/min and from 6 mL/min to 4.4 mL/min for the single and 4-spiral device, respectively. Symbol represents the position of peak intensity of a fluorescent streak. Error bar represents the width of fluorescent streak (threshold: 15% above normalized background intensity).

**Supplementary Table 1** Estimation of reduction of dead cells during perfusion culture based on Monod growth kinetics

According to biomass balance,

Rate of dead cell accumulation = rate of dead cell generation – rate of dead cell removal

$$V \times dX = \mu \times dt \times X \times V - \alpha \times X \times F \times dt$$

$$\leftrightarrow \frac{dX}{dt} = \mu X - \alpha \times \frac{F}{V} \times X = \left(\mu - \alpha \times \frac{F}{V}\right) \times X$$

where $V$ is working volume of the bioreactor; $X$ is cell concentration in the bioreactor (million cells/mL); $\mu$ is death rate ($hr^{-1}$); $t$ is time (hr); $\alpha$ is relative ratio of the cell concentration in the perfusate (removed stream) to that in the bioreactor (*e.g.*, chemostat when $\alpha = 1$); $F$ is volumetric flow rate for the perfusate (removed stream; volumetric flow rate (mL/hr) for the outer outlet of the device).

Assuming $\mu = 0.02\ hr^{-1}$ (typical growth rate is $0.03\ hr^{-1}$ for CHO cells), $V = 350$ mL, and $F = 700$ mL/day = 29.2 mL/hr,

$$\frac{dX}{dt} = (0.02 - \alpha \times 0.08) \times X$$

| Figure | Single-pass dead cell removal efficiency (%) | $\alpha$ | Initial dead cell concentration (million cells/mL) | Time elapsed (hr) | Final dead cell concentration (million cells/mL) | Dead cell reduction compared with no dead cell removal (%) |
|---|---|---|---|---|---|---|
| N/A | 0 | 0 | $X_0$ | 96 | $6.82X_0$ | 0 |
| 5c and 5d | 3.5 | 0.15 | $X_0$ | 96 | $2.16X_0$ | 68.3 |
| 5c and 5d | 6.1 | 0.23 | $X_0$ | 96 | $1.17X_0$ | 82.8 |
| 5c and 5d | 14.2 | 0.48 | $X_0$ | 96 | $0.17X_0$ | 97.5 |
| 5c and 5d | 14.2 | 0.48 | $X_0$ | 192 | $0.03X_0$ | 99.6 |
| 5c and 5d | 20.1 | 0.60 | $X_0$ | 96 | $0.07X_0$ | 99.0 |