Membrane-less microfiltration using inertial microfluidics

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Supplementary information

Table S1. Head to head comparison of inertial filtration system and existing techniques available in the industry for cell retention from perfusion bioreactors.

Technique/ Criteria	Filtration	Centrifugation	Hydrocyclones	Gravity sedimentation	Ultrasound/ electrophoresis	Inertial filtration system
Cell viability (%)	50-90	70-85	80-85	88-100	70-95	95
Throughput*	Medium	High	High	Low	Low	High
Running cost	High	High	Low	Low	Very High	Low
Scalability	Good	Good	Fair	Poor	Poor	Good
Separation efficiency (%)	63-95	95-100	>85	>85	>95	>95
Cell Concentration (10^6 cells /mL)	Typically 3-30	3-17	3	3-15	20-50	Up to 50
Advantages	Applicable to all cell types	High separation efficiency	Low cost, continuous processing	Low cost, high cell viability	High separation efficiency	Low cost, Continuous processing, High separation efficiency
Disadvantages	Low viability, clogging	High capital cost	Not applicable to smaller cells	Too slow	Low throughput, High capital cost	- - -

*High: tens to hundreds of mL/min range, Medium: hundreds of µL/min to mL/min range, low: tens of µL/min range.

Table S2. Reynolds' number calculation for different trapezoidal channels used in this study.

	Hydraulic diameter/ µm	Kinematic viscosity (of water/PBS)/ m^2/s	Flow Velocity ($\times 10^{-9}$ m^3/s)	Channel Reynolds' Number
CHO cells (Bioreactor; channel dimensions: 80×130 µm)	89.84	1×10^{-6}	100.2 (=6 mL/min)	283.751
CHO cells (Cell cycle synchronization; channel dimensions: 80×130 µm)	178.460	1×10^{-6}	16.7 (=1 mL/min)	47.292
Yeast cells (channel dimensions: 30×70 µm)	178.460	1×10^{-6}	33.4 (=2 mL/min)	133.323

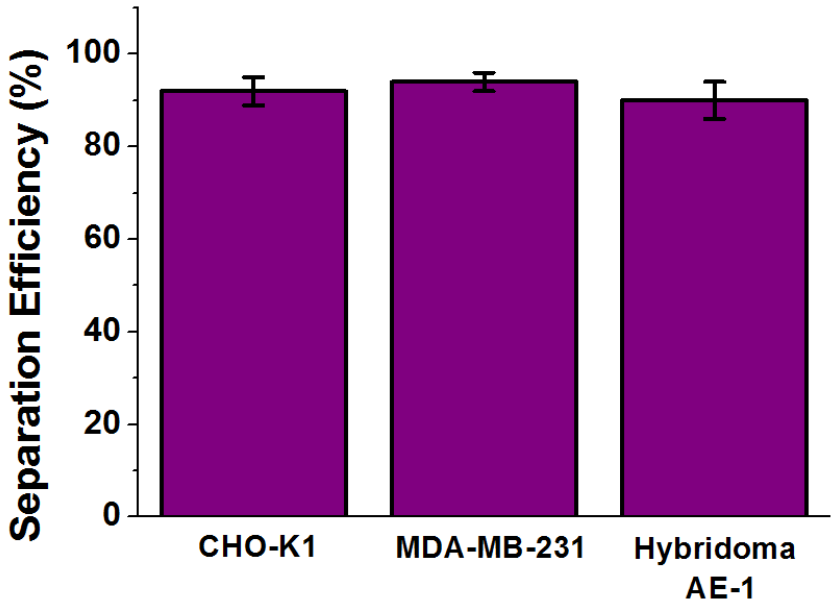

Figure S1. Recovery efficiency of inertial microfiltration system for processing of three different cell lines at concentration of 1×10^7 cells/mL.

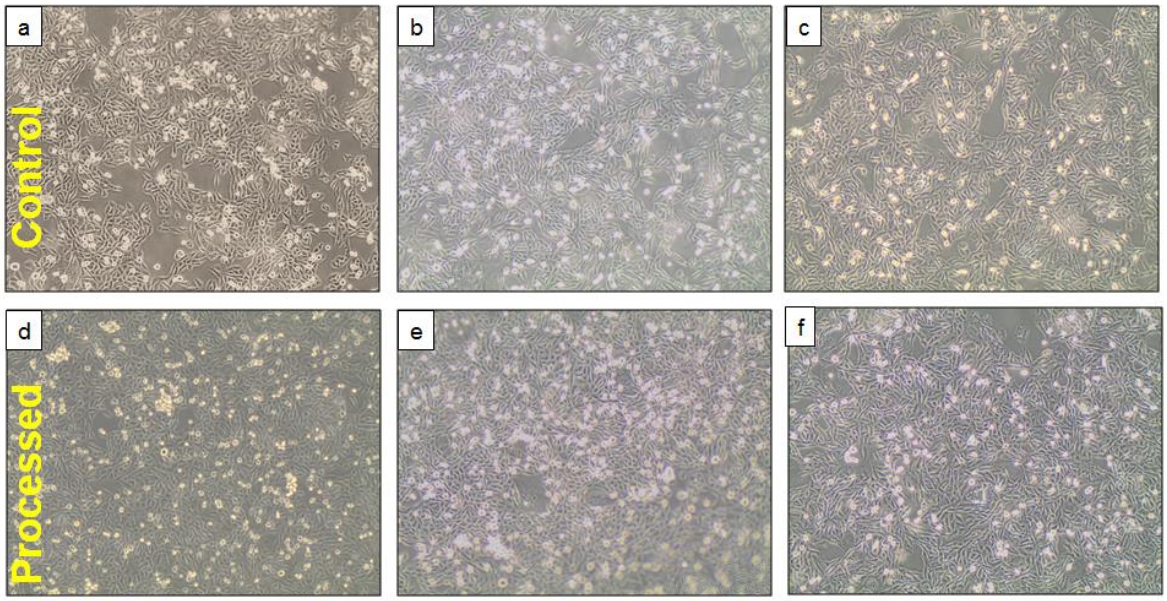

Figure S2. Phase contrast micrographs of cultures of control (unsorted) CHO cells (a-c) and sorted cells (d-f) by the inertial microfiltration system. The images indicate no significant differences between the morphology and proliferation rate of the cells suggesting high viability and sterility.

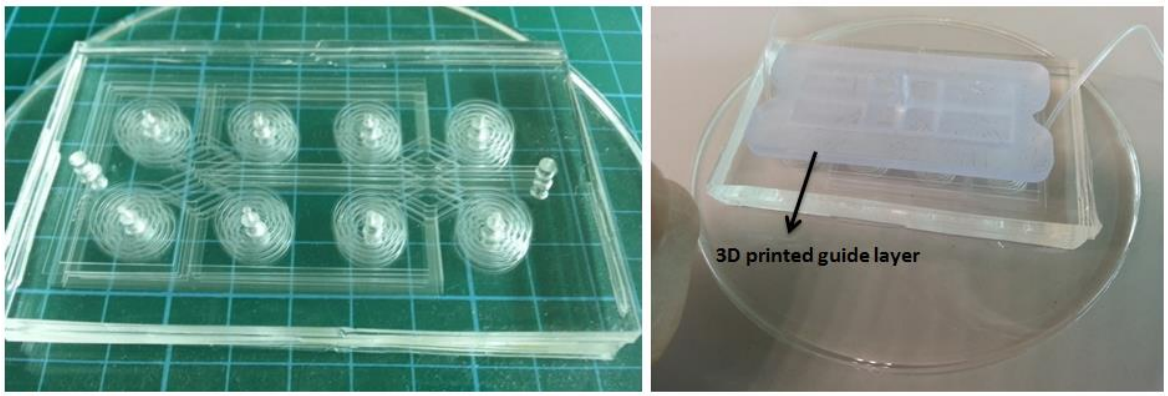

Figure S3. Optical image of a multiplexed inertial microfiltration system designed for separation of yeast cells. Similar to our system developed for mammalian cell retention, this system also consists of multiple layers of PDMS sheets with embossed microchannels bonded together for continues cell retention from large sample volumes. The microchannels used in this system has also trapezoidal cross-section with 30×70 µm channel dimensions.

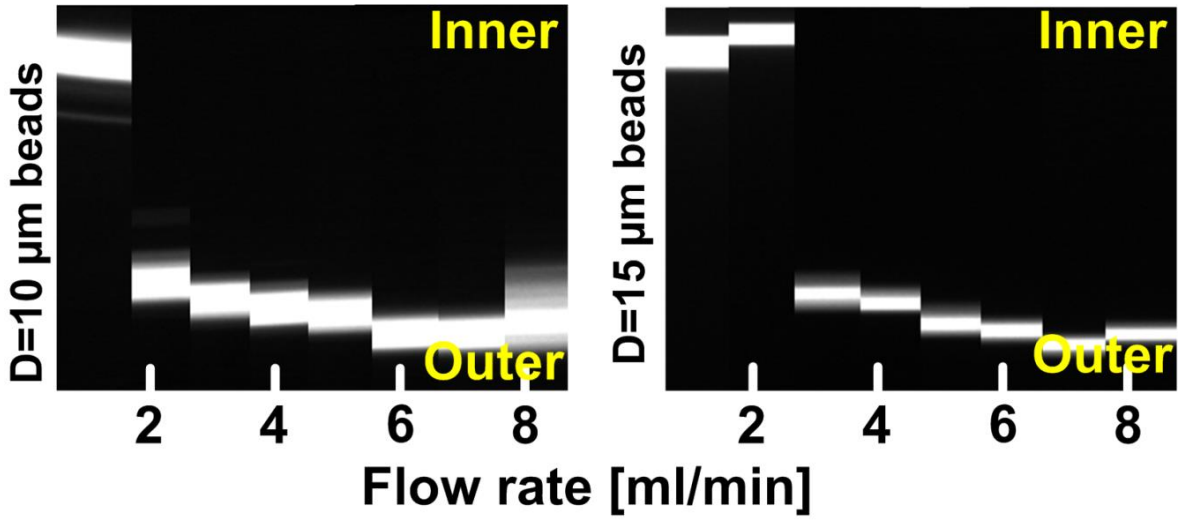

Figure S4. Top-view experimental observation of fluorescently microparticles at the outlet of a spiral device with trapezoidal cross-section (80×130 µm) used in this study for separation and fractionation of mammalian cells. In can be seen that at the flow rate of 6 mL/min, both 10 and 15 µm particles can be focused near the outer wall (i.e., filtration mode of our system) while at the 2 mL/min flow rate, the 10 µm beads are focused near the outer wall and 15 µm beads are focused near the inner wall (i.e., fractionation mode of our system).

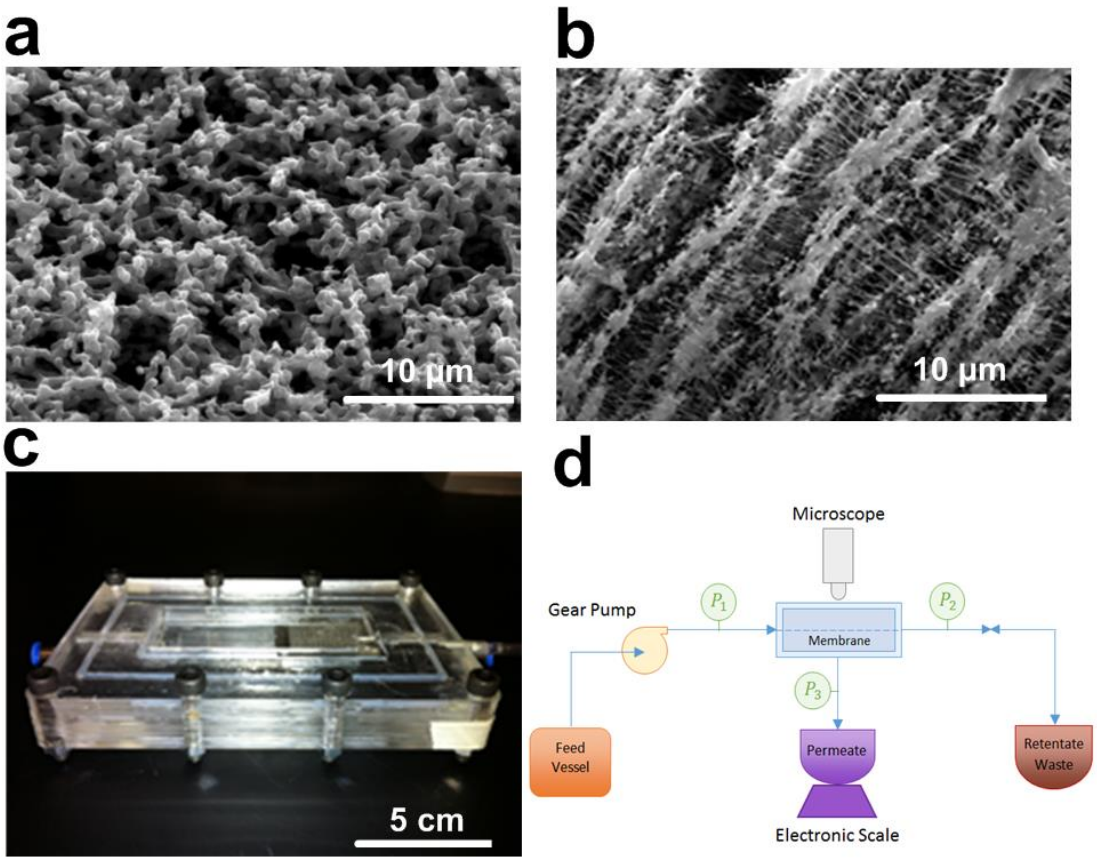

Figure S5. (**a**) & (**b**) SEM photos of the Cellulose (Millipore, Cat. No. AAWP04700) and Teflon filter (Pall, Cat. No. P5PL047) with nominal pore size of around 1 µm used in this study for yeast separation. (**c**) Optical image of the cross-flow filtration setup made from PMMA and used for microfiltration tests. (**d**) Schematic representation of the experimental setup used for the tests.

SI Movie Legends

Movie S1
High speed video (6400 fps) illustrating the separation of CHO cells at concentration of 10^6 cell/mL using a single spiral channel at flow rate of 6 mL/min. Focused CHO cells (near the outer wall (bottom side)) are exiting the system from the outer outlet (i.e., can be returned to the cell culture flask or bioreactor) while clarified culture medium is collected continuously via the inner outlet.

Movie S2
High throughput cell separation (100 mL/min) using a multiplexed inertial microfiltration system (20 spiral channels).

Movie S3
High speed video (6400 fps) illustrating the separation of yeast cells at concentration of 0.1 gr/l using a single spiral channel at flow rate of 2 mL/min. Focused yeast cells (near the inner wall (bottom side)) are exiting the system from the outer outlet while clarified culture medium is collected continuously via the outer outlet. At this concentration, around 10-12% of yeast cells were escaping to the outer outlet (i.e., filtrate) during the 1^{st} cycle of enrichment; however, the filtrate can be further purified through 2^{nd} cycle of processing.

Movie S4
High speed video (6400 fps) illustrating the fractionation of CHO cells at concentration of 10^6 cell/mL using a single spiral channel at flow rate of 1.5 mL/min. It can be seen that smaller CHO cells are trapped inside the Dean vortices and remained near the outer wall while bigger cells are focused near the inner wall, exiting the device from both outlets.