INERTIAL MICROFLUIDIC VORTEX SORTER FOR CONTINUOUS ISOLATION OF RARE CELLS FROM BLOOD WITH 15,000× ENHANCED PURITY
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ABSTRACT
This work presents an inertial microfluidic device for continuous size-based sorting and purification of cells with significantly enhanced purity and high efficiency. The device uses microvortex-based sorting and integrates two units by connecting the high-pass outlets of the first unit into the inlet of the second. Using this approach, we demonstrated continuous double sorting and purification of rare large particles from blood with 15,000× enhanced purity and high efficiency. We envision this inertial microfluidic sorter can serve as a versatile tool for sorting of cells from complex cellular mixtures for cell biology research and clinical diagnostics.

KEYWORDS: Inertial microfluidics, cell sorting, cancer cell isolation

INTRODUCTION
Sorting of target cells with high purity from complex cellular samples is challenging yet essential for downstream cell biology research and clinical diagnostics. It becomes even more challenging for processing samples containing only a small fraction of target cells in large quantities of background cells and such as isolation of circulating tumor cells (CTCs) from human blood (1~10/mL for CTCs and 5×10^9/mL for red blood cells) [1].

Inertial microfluidics has achieved size-based sorting of cells with throughput orders of magnitude higher than other sorting techniques [2,3]. Although most inertial microfluidic devices demonstrate sorting of microbeads or cells with promising efficiency of >90%, it is still far from sufficient to get highly purified target cells from cell mixture due to several orders of magnitude differences in cell concentrations. Herein, we introduce an integrated vortex-based [4,5] inertial microfluidic chip for continuous double sorting and purification of biomicroparticles with high efficiency and significantly enhanced purity.

Our design consists of a straight microchannel followed by two sorting microchambers for sequentially focusing and sorting of large microparticles from smaller ones (Fig.1). As shown in Fig.1b, microparticles are first ordered into two bands along microchannel sidewalls undergoing inertial lift forces. Downstream, vortices are generated in the first sorting...
unit for sorting of larger microparticles from smaller ones into different outlets. To further remove the remaining smaller particles and sort the large cells, we designed a second sorting unit downstream from the side outlets of the first unit. By accelerating flow with buffer, we created proper flow conditions for the second unit to sort large rare cells into outlet 3 (O3), while medium cells and RBC impurities flow into outlet 2 (O2).

Using large particles as target cell surrogates, we demonstrated continuous sorting of large target particles from blood with high efficiency, 15,000× enhanced purity and a throughput of ~3.8×10^6 cells/min. With this functionality, the inertial microfluidic sorter can become a powerful candidate for size-based sorting and purification of cellular samples in cell biology and clinical diagnostic applications.

**EXPERIMENTAL**

We used standard soft lithography process to fabricate microchannels in polydimethysiloxane (PDMS). We first loaded a syringe with microparticle or blood samples and connected it to the device by using a 1/16” tubing (Upchurch Scientific) with proper fittings (Upchurch Scientific). We pumped samples into devices with designed flow rate using a syringe pump (Legota 180, KD scientific). We used an inverted epi-fluorescence microscope (IX71, Olympus Inc.) equipped with a 12-bit high-speed CCD camera (Retiga EXi, QImaging) to take bright-field images. We set the exposure time to minimum value (10 µs) and sequentially took 200 images with minimum time interval. By stacking images in ImageJ, we established a complete view of particle motion.

**RESULTS AND DISCUSSION**

A fluidic circuit model was used to aid the device development (Fig.2). We designed a pair of symmetric 250×500µm^2 microchambers with flow resistance ratio R_2/R_1=14 as the first unit. Using Q_sample=190µL/min, this chamber has separation cutoff of ~13µm. Based on analogous electric circuit model, the total flow rate from side outlets is Q_1= ~25µL/min. To enable proper separation at the second unit, we introduce buffer at Q_buffer=90µL/min to accelerate the flow to ~115µL/min. At this flow rate, we designed R_5/R_4=10 to set a cut-off size of 20µm for the second unit. The cutoff of each chamber can be modified by adjusting the flow rates or the resistance ratios of the sorting units. The flexibility and convenience of tuning the sorting cutoffs make the device viable for sorting cellular samples with different size distributions without the burden of redesigning and refabrication of the devices.

To demonstrate feasibility of sorting rare large cells from blood, we spiked 23µm and 18µm diameter beads (to mimic large rare cells) into diluted human blood. The resulting concentrations were 700/mL and 18,000/mL for 23µm and 18µm diameter beads correspondingly in ~1.5×10^7/mL red blood cells (RBCs). As Fig.3a shows, majority of RBCs are extracted in O1 of the first unit, while the 23µm and 18µm beads are sorted to side outlets together with the remaining RBCs. The product from the side outlets is sorted again in the second sorting unit, in which the 18µm microbeads and the remaining RBCs are separated into O2. The rare 23µm beads are extracted from O3. The bright-field images (Fig.3b) illustrate successful isolation of 23µm beads with high purity. Quantitative measurements of concentration (Fig.3c) and purity (Fig.3d) indicate significant decrease of RBC concentration by 15,800× and increase of 23µm bead purity from 0.005% to 71% (~15,000×) in O3. Moreover, the separation efficiencies for the 23µm, 18µm beads and RBCs are ~99%, ~99%, and ~94% respectively, indicating efficient size-based sorting using...
this device (Fig.3e). The sorting efficiency of RBCs from O1 can be further improved by fabricating the device into rigid epoxy to prevent channel expansion.

CONCLUSION

In conclusion, we developed an inertial microfluidic sorter for double extraction and purification of large target cells from massive smaller background cells with significantly enhanced purity and high efficiency. We envision this device platform as a powerful technique for isolation and purification of rare cellular components (e.g., CTCs) from complex sample matrices for cell biology and clinical diagnostic applications.

REFERENCES


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