Supporting Information

Integrated DEP presorting and wireless electrode array for high-throughput selective single-cell isolation

Thilini N. Rathnaweera, Dhatchayani Rajkumar and Robbyn K. Anand

Department of Chemistry, Iowa State University, Ames, Iowa 50011, United States

Materials and Methods

Device geometry and dimensions. In this work, all the device features (including channels, chambers, leak channels, and micropockets) were 25 μm tall as confirmed by profilometry. We employed microchannels with varying widths from 0.2-0.8 mm, and their performance was compared to the previously established design with a channel width of 0.1 mm.[1] In wider channel designs, the number of parallel channels employed was two and each parallel channel was 13.3 mm long. The two channels were connected via bifurcated to share a common inlet and an outlet. Along the length of each channel, 20 microchambers were uniformly distributed, with 10 chambers on either side, leading to a total of 40 microchambers per chip. Each chamber measured 200 μm wide x 400 μm deep and was connected to the channel via a micropocket 20 μm wide and 20 μm deep. Each chamber was also connected to the channel via a 30˚-inclined leak channel (measured from the chamber wall) which is 7 μm wide and 300 μm long. All other design features including the inter-channel distance and inter-chamber distance were kept constant from our previous work.[1,2]

In the 0.8 mm-wide channel, a hydrodynamic module was created to focus cells prior to isolation. This module consists of eight horizontal tracks each with a width of 50 μm and a length of 1.0 mm. The tracks were separated with 20 μm-wide PDMS barriers. The front edge of the horizontal track was curved towards the center of the channel,

The electrode design of this work consisted of two distinct modules that were positioned sequentially. At the downstream end of the chip (left side, **Figure 1**) the cell-isolation module or the BPE array consisted of rectangular BPEs ("bar electrodes") that are 0.5 mm wide and 6.12 mm long aligned to intersect all chambers in a row such that the distance from the inner edge of the

micropocket to the leading edge of the electrode was 300 μm. This electrode alignment was optimized in our recently published work.[1] 150 μm upstream of this module (middle segment of **Figure 1**) was a cell-redirection module. For redirection, either single or multiple "chevron-shaped" electrodes were fabricated with varying inclinations of 45, 60 and 75° (measured from the vertical plane). Each chevron was 30 μm wide, and among multiple chevrons, the gap was maintained at 10 μm. The pointed tip of the chevron was curved with at least a curvature radius of 10 μm to avoid potential cell damage. The two electrode modules were operated independently – a distinct voltage and frequency was applied to each via a pair of driving electrodes connected to external wire leads.

Device fabrication. Electrodes used in this work were fabricated with standard photolithography and etching techniques. First the thin-film gold electrodes (bars and chevrons) were patterned on Au-coated slides (100 nm-thick Au film adhered on 5 nm Cr film, Omega Optical, Brattleboro, VT) using positive photoresist (AZP4620, Integrated Micro Materials (IMM), Argyle, TX). A plastic photomask (FineLine Imaging, Colorado Springs, CO) was used for patterning followed by wet etching, first in AZ 400K developer (IMM, Argyle, TX), second in Au etchant (10% KI/2.5% I_2), third in Cr etchant (Sigma-Aldrich, St. Louis, MO) and finally in acetone. Patterned-Au electrodes were cleaned with basic piranha (1:1:1 ammonium hydroxide, hydrogen peroxide and Type 1 water) for at least 15 min at 95 ºC prior to assembly.

The channels and chambers were fabricated using standard soft lithography techniques. Briefly, an SU-8 master mold was fabricated by patterning negative photoresist, SU-8 2025 (MicroChem Corp., MA) on a 4-inch Si wafer followed by a soft bake and UV exposure (mask aligner, ABM-USA, San Jose, CA) through a photomask (Cr-coated glass (Front Range

Photomask, Las Vegas, NV)). Following a post-exposure bake, the wafer was developed in SU-8 developer (MicroChem Corp., MA) to yield the master mold. PDMS replicas were then cast-molded with 10:1 ratio of elastomer to crosslinking agent. The PDMS was cured for at least 72 h at room temperature before patterned monoliths were cut out and peeled from the mold. Then inlets (3.0 mm) and outlets (1.0 mm) were punched using biopsy punches. The monoliths were thoroughly cleaned with 200-proof ethanol (Decon Labs, King of Prussia, PA).

Both the thin-film electrode and PDMS monolith were dried with a stream of N_2 gas and then plasma treated (Harrick Plasma, Ithaca, NY) at high power for 30 s for surface activation. Following plasma treatment, the PDMS channel design was manually aligned to the electrode design under a microscope. The assembled device was baked in an oven overnight at 65 ºC, to ensure irreversible bonding.

Table S1. Increasing channel widths tested as compared to established 100-µm wide channel, design parameters, applied voltages for cell capture and simulated electric fields within the micropockets.

Channel width (µm)	Flow rate (nL/min)	Avg. linear velocity (µm/s)	No. of parallel channels	Lead-to-lead distance (mm)	Applied voltage (V_{p-p})	Avg. electric field (kV/m)
100	100	666	4	3.89	66	92
200	200	666	2	1.98	34	92
400	400	666	2	2.38	36	92
600	600	666	2	2.78	38	92
800	800	666	2	3.18	40	92

Table S2. The dielectric properties of healthy blood-derived PBMCs and cultured BCs as measured by the 3DEP instrument.

Dielectric property	Cell type	
	PBMCs	BCs
Cytoplasm Conductivity (*S/m)*	0.11 ± 0.10	0.17 ± 0.06
Relative cytoplasm Permittivity	60	60
Specific Membrane Conductance *(S/m^2)*	739.6 ± 373.1	505.4 ± 37.90
Specific Membrane Capacitance *(F/m^2)*	0.005 ± 0.001	0.011 ± 0.003

Table S3. Summary of experimental conditions used for isolation of BCs from background PBMCs in an 800 µm-wide channel as compared to the optimized 100 µm-wide channel.

Channel width (µm)	Frequency (kHz)	Voltage (Vp-p)	Simulated avg. EF (kV/m)	Flow rate (nL/min)	Avg. linear velocity (µm/s)	No. of parallel channels
100	45	75	110	50	330	4
800	45	52	110	400	330	2

Table S4. Countess™ data of three independent trials showing the cell concentration, viability and diameter of healthy blood-derived PBMCs and cultured BCs after fluorescent labeling.

Cell type	Trial #	Cell concentration (mL^{-1})	Viability (%)	cell diameter (µm)
PBMCs	1	2.3×10^7	87	8.96
	2	2.34×10^7	86	9.41
	3	1.88×10^7	88	9.08
	Avg.	2.17×10^7	87	9.15
MDA-MB-231 cells	1	3.6×10^5	92	21.74
	2	4.0×10^5	96	20.77
	3	3.0×10^5	94	20.92
	Avg.	3.5×10^5	94	21.14

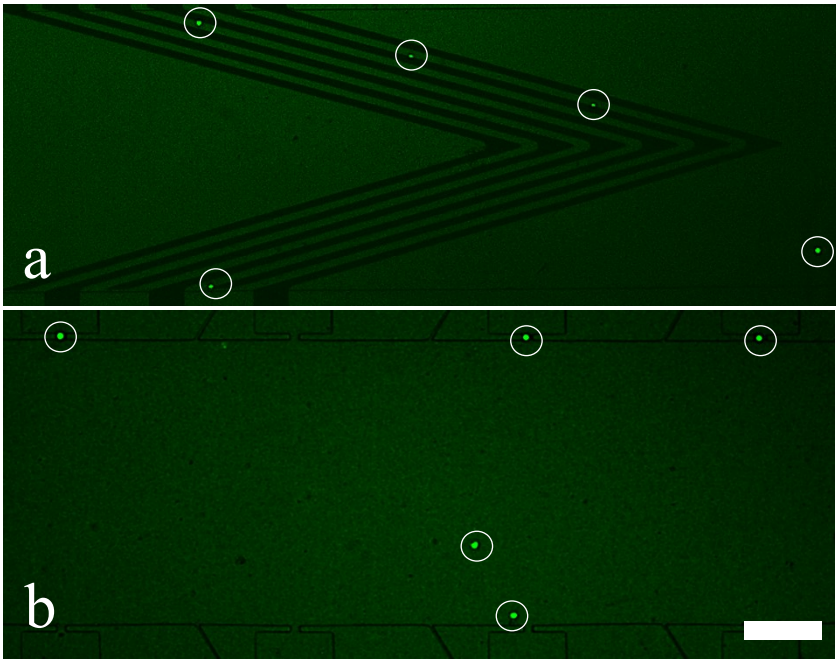

Figure S1. Fluorescence micrographs showing live cells stained with Calcein AM (a) flowing through the channel, redirected by interdigitated guide electrodes inclined at 75° and (b) held in the micropockets 20 min after capture in the SC-DEPOT device. Scale bar, 200 µm.

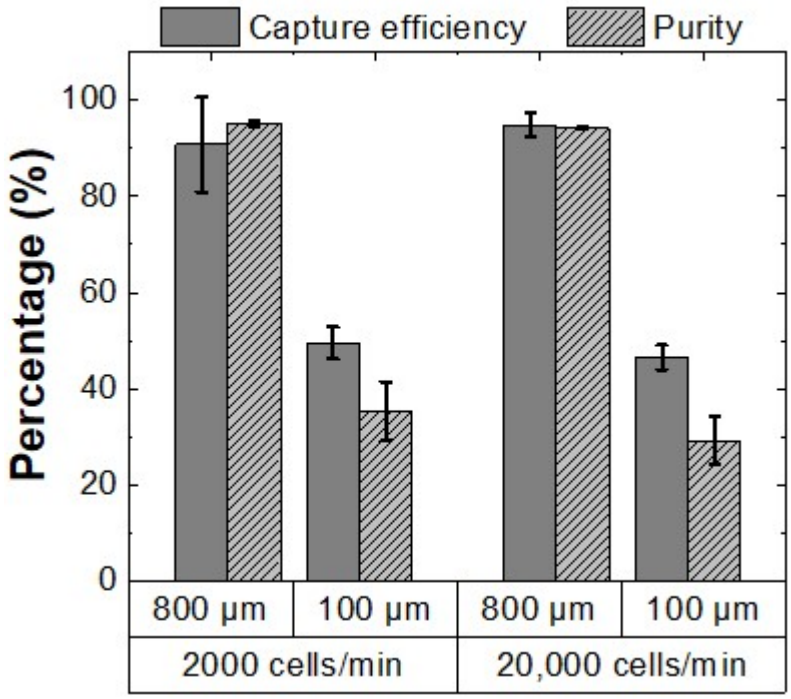

Figure S2. Bar graph showing the comparison of capture efficiency and purity of MDA-MB-231 cells isolated from PBMC backgrounds at matched throughput per channel for 800 µm-wide (SC-DEPOT) and 100 µm-wide (iDEP) devices.

References

(1) Rathnaweera, T. N.; Anand, R. K. IDEP-Based Single-Cell Isolation in a Twodimensional Array of Chambers Addressed by Easyto-Align Wireless Electrodes†. *Lab on chip* **2025**, *25* (Dld), 1600–1610. https://doi.org/10.1039/d4lc00976b.

(2) Li, M.; Anand, R. K. Integration of Marker-Free Selection of Single Cells at a Wireless Electrode Array with Parallel Fluidic Isolation and Electrical Lysis. *Chem Sci* **2019**, *10* (5), 1506–1513. https://doi.org/10.1039/c8sc04804e.