Supporting information for

Highly efficient combination of multiple single cells using deterministic single-cell combinatorial reactor

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Supplementary Movie Legends

Supplementary Movie 1. Selective trapping of single cells using addressable electroactive trap-wells. The selected SiO_2 trap-wells, indicated by yellow dotted circles, were activated by applying an electrical potential of 10 Vpp at 8 MHz to the electrodes at t = 7 sec. The cells passing over the activated trap-wells were efficiently trapped by DEP force. However, cells that passed over deactivated wells were not trapped. Once the single cells were trapped into the SiO_2 trap-wells, a second cell cannot be trapped into the same trap-wells, allowing highly efferent single-cell trapping.

Supplementary Movie 2. Selective release of trapped single cells. After trapping PC3 cells by activating all SiO_2 trap-wells with an electrical potential of 10 Vpp at 8 MHz, selected trap-wells, indicated by grey dotted circles, were deactivated at t = 14 sec, and the cells in those wells were immediately released. However, cells in the still-activated trap-wells, indicated by yellow dotted circles, were not released.

Supplementary Movie 3. Representative PC3 cell trapping using DSCR. The suspension of PC3 cells was introduced into the microfluidic channel at a flow rate of 4 μL min^{-1}. PC3 cells were trapped into the trap-well 1 by applying a 15 Vpp sinusoidal electric potential at 8 MHz to the electrode 1.

Supplementary Methods

When a dielectric particle is subjected to a non-uniform electric field, dielectrophoresis (DEP) force is exerted on the particle. The DEP force ($\boldsymbol{F}_{DEP}$) acting on the spherical cell of a radius (a) can be approximated by

$$\boldsymbol{F}_{DEP} = 2\pi\epsilon_e a^3 Re[K(2\pi f)]\nabla|\boldsymbol{E}|^2$$

where ϵ_e and $\boldsymbol{E}$ are permittivity of suspending medium and applied electric field, respectively. Clausius-Mossotti (CM) factor, $K(2\pi f)$, which represent the relative permittivity between the cell and the suspending medium, for the spherical shell model[1] is

$$K(2\pi f) = \frac{\epsilon^*_{cell} - \epsilon^*_e}{\epsilon^*_{cell} + 2\epsilon^*_e}; \quad \epsilon^*_{cell} = \epsilon^*_m \frac{a^3(\epsilon^*_i + 2\epsilon^*_m) + 2(a-d_m)^3(\epsilon^*_i - \epsilon^*_m)}{a^3(\epsilon^*_i + 2\epsilon^*_m) - (a-d_m)^3(\epsilon^*_i - \epsilon^*_m)}$$

where $\epsilon^* = \epsilon + \frac{\sigma}{2\pi f}j$ is complex permittivity, and $j = \sqrt{-1}$. σ, f and d_m are conductivity, frequency of the applied electric potential, and thickness of the cell membrane, respectively. Subscripts $cell$ and e represent cell and the suspending medium, and i and m represent internal and membrane of the cell, respectively.

When a cell is exposed to an external a.c. electric field, a transmembrane potential is induced. The maximal induced transmembrane potential[2], $V_{tm}(f)$ is

$$V_{tm}(f) = 1.5a|\boldsymbol{E}|\left[1 + \frac{G_s}{\sigma_e a} + \frac{a}{d_m}(\sigma_m + 2\pi f\epsilon_m j)\left(\sigma_i + \frac{1}{2\sigma_e} + \frac{G_s}{\sigma_e\sigma_i a}\right)\right]^{-1}$$

where G_s represents surface conductance. If V_{tm} exceeds a critical value, permeable pores are formed on the cell membrane, which could induce electroporation of the cell membrane.

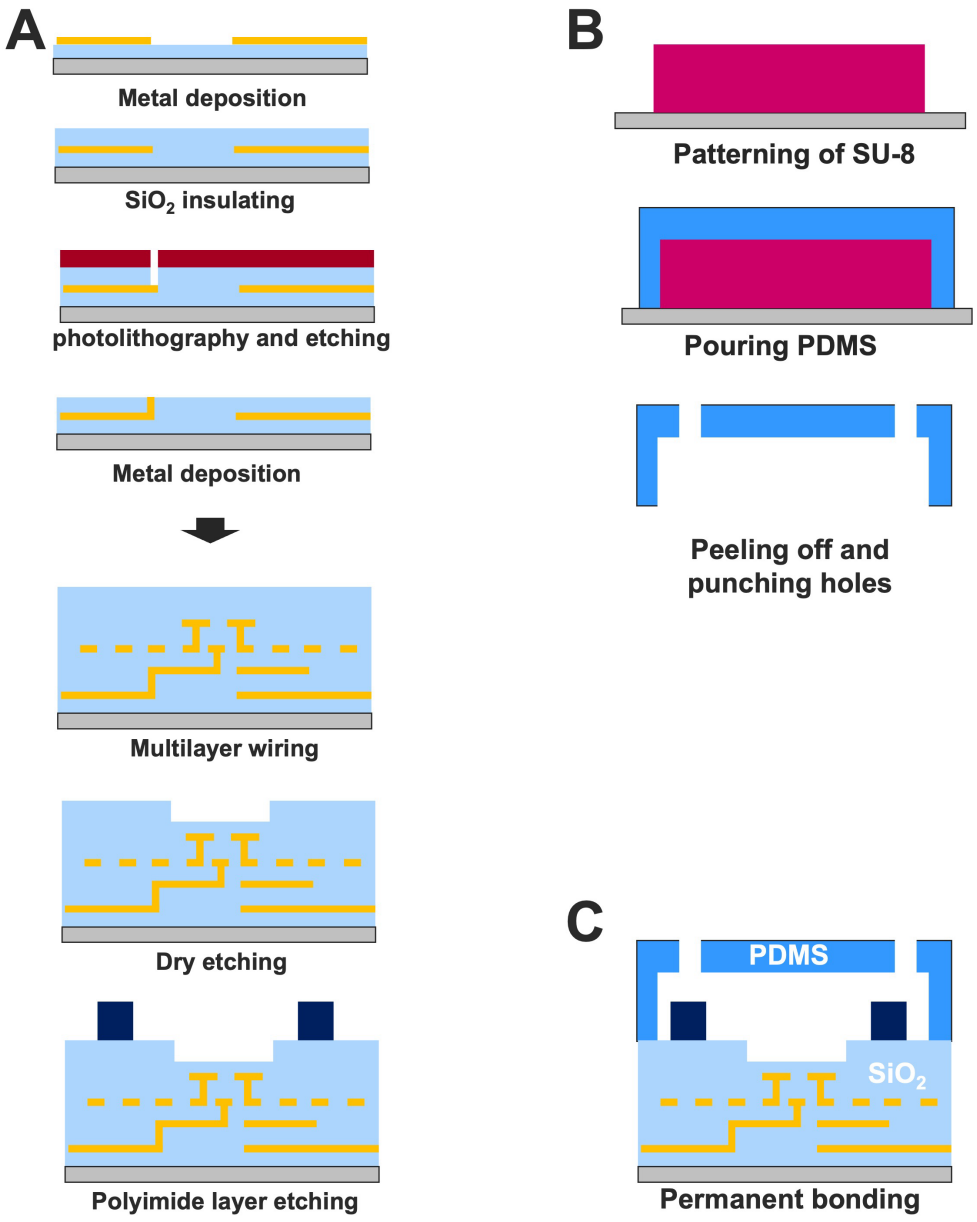
To determine proffer frequency of the electric field, relative DEP force, $Re[K(2\pi f)]$, is compared with relative transmembrane potential $\left[1 + \frac{G_s}{\sigma_e a} + \frac{a}{d_m}(\sigma_m + 2\pi f\epsilon_m j)\left(\sigma_i + \frac{1}{2\sigma_e} + \frac{G_s}{\sigma_e\sigma_i a}\right)\right]^{-1}$ as shown in Supplementary Fig. S2 with typical cell parameters of mammalian cells (Supplementary Table).

Supplementary Table

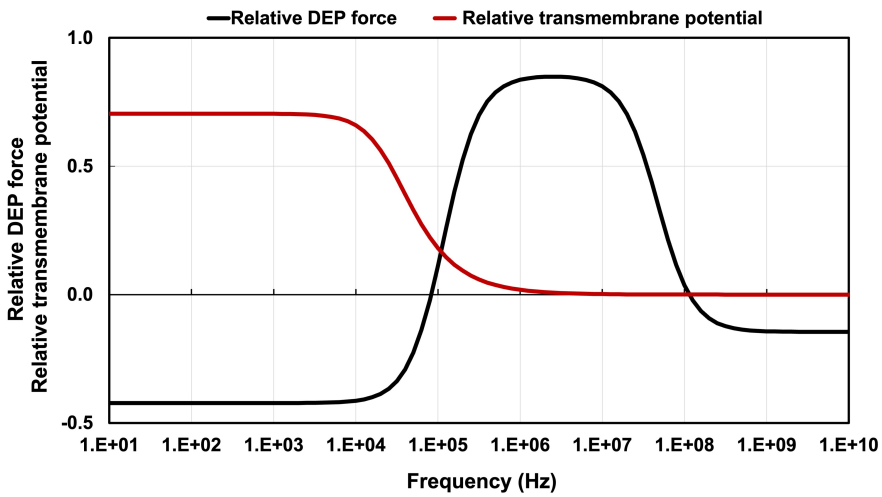
Supplementary Table. Typical cell parameters[3] used for the calculation.

		Unit	Internal (i)	Membrane (m)	External (e)
Conductivity	σ	S m^{-1}	0.5	3×10^{-6}	0.02
Permittivity	ϵ	ϵ_0	50	8	80
Surface conductance	G_s	S m^{-2}	10^{-9}		
Membrane thickness	d_m	m	8×10^{-9}		
Cell Radius	a	m	7.5×10^{-6}		

Supplementary Figures



Supplementary Fig. S1. Schematic illustration of device fabrication. (A) A Si/SiO2 structure fabrication using the back-end of line process. (B) Fabrication of PDMS microfluidic channel. (C) Integration of the Si/SiO2 structure and PDMS microfluidic channel.



Supplementary Fig. S2. Estimated frequency dependence of relative DEP force ($Re[K(2\pi f)]$) and relative transmembrane potential ($\left[1+\frac{G_S}{\sigma_e a}+\frac{a}{d_m}(\sigma_m+2\pi f\epsilon_m j)\left(\sigma_i+\frac{1}{2\sigma_e}+\frac{G_S}{\sigma_e\sigma_i a}\right)\right]^{-1}$).

Supplementary References

1. T. B. Jones, *Electromechanics of particles*, Cambridge University Press, Cambridge ; New York, 1995.
2. D. R. Albrecht, G. H. Underhill, T. B. Wassermann, R. L. Sah and S. N. Bhatia, *Nature methods*, 2006, **3**, 369-375.
3. G. Fuhr, H. Glasser, T. Muller and T. Schnelle, *Biochim Biophys Acta*, 1994, **1201**, 353-360.