SUPPORTING MATERIALS

CFD simulation to estimate the cell location in each DEP-well

The dielectrophoretic force (F_{DEP}) acting on a cell with radius, r, as the result of an applied AC field of frequency, ω, according to the effective dipole moment approach, is given:

$$F_{DEP} = 2\pi\varepsilon_m r^3 [\mathrm{Re}\,(f_{CM}(\omega))\nabla E^2_{RMS}] \quad \text{(S Eq. 1)}$$

where E_{RMS} is the RMS value of the field strength, $\mathrm{Re}(f_{CM})$ is the real component of the Clausius-Mossotti (CM) factor, which determines the direction of the DEP force either to attract (positive DEP) or repel (negative DEP) cells to high electric field regions, and ε_m is the permittivity of the medium.

The CM factor (f_{CM}) of a particle or cell can be expressed as:

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad \text{(S Eq. 2)}$$

$$\text{and} \qquad \varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \quad \text{(S Eq. 3)}$$

where ε_p^* and ε_m^* are the complex permittivities of the particle/cell and medium respectively, ε is the permittivity and σ is the conductivity of the particle/cell or medium, and j is $\sqrt{-1}$.

For a mammalian cell lacking a cell wall, a single shell model can be used to describe the complex permittivity (ε^*_{p-eff}), which is an effective value combining the influence of the cell membrane and cell cytoplasm, thus

$$\varepsilon^*_{p-eff} = \varepsilon^*_{mem} \frac{\left(\frac{r}{r-\delta}\right)^3 + 2\frac{\varepsilon^*_{cyto} - \varepsilon^*_{mem}}{\varepsilon^*_{cyto} + 2\varepsilon^*_{mem}}}{\left(\frac{r}{r-\delta}\right)^3 - \frac{\varepsilon^*_{cyto} - \varepsilon^*_{mem}}{\varepsilon^*_{cyto} + 2\varepsilon^*_{mem}}} \quad \text{(S Eq. 4)}$$

where subscripts *mem* and *cyto* correspond to the cell membrane and cytoplasm, respectively and δ is the thickness of the cell membrane, assumed to be 7 nm for most cells. Thus, the CM factor can be expressed as:

$$f_{CM}(\omega) = \frac{\varepsilon^*_{p-eff} - \varepsilon^*_m}{\varepsilon^*_{p-eff} + 2\varepsilon^*_m} \quad \text{(S Eq. 5)}$$

The gravitational force (F_{grav}) on the cells is given as:

$$F_{grav} = (4/3)\pi r^3(\rho_m - \rho_{cell})g \quad \text{(S Eq. 6)}$$

where ρ_m and ρ_{cell} are the densities of the medium and cells, respectively, and g is the gravitational acceleration constant.

When the applied frequency creates a negative DEP force, the cells will be levitated to a equilibrium position so that the induced DEP force and the gravitational force are in balance and the net force acting on the cells in the vertical direction is zero, and

$$2(\rho_m - \rho_{cell})g = 3\varepsilon_m[\mathrm{Re}(f_{CM})\nabla E^2_{RMS}] \quad \text{(S Eq. 7)}$$

However, when the applied frequency creates a positive DEP force, the gravitational force and DEP force are in the same direction and the cell's final position will be on the top of the electrodes.

By taking into account both the induced DEP force (related to the CM factor) and the gravitational force, the position of the cells in the electric field can be calculated and used to determine the strength of the electric field to which the cells are exposed.

SUPPORTING TABLES

Table S1: Parameter values used for HuNSPC SC27

Parameter	Denotation	Value	Unit	Source
Electric constant	ε_0	8.85E+12	F/m	
Gravitational acceleration constant	g	9.8	m/s^2	
Medium conductivity	σ_m	0.011	S/m	Measured
Medium relative permittivity	ε_m	80	ε_0	[1]
Membrane conductivity	σ_{mem}	4.50E-06	S/m	[1]
Membrane relative permittivity	ε_{mem}	6.01	ε_0	[1]
Cytoplasmic conductivity	σ_{cyto}	0.72	S/m	[1]
Cytoplasmic permittivity	ε_{cyto}	60	ε_0	[1]
Radius of cell	r	7.15 e-6	m	Measured
Thickness of cell membrane	δ	7e-9	m	[1]
Cell density	ρ_{cell}	1.04-1.1	kg/m^3	[2]
DEP buffer density	ρ_m	1026.52	kg/m^3	Measured

Table S2: Parameter values used in simulating particle deflection

Parameter	Denotation	Value	Unit
Channel inlet velocity		5.55E-3	m/s
Channel outlet pressure	p	0	N/m^2
Buffer viscosity		8.85E-4	Kg/m-s
Buffer density	ρ_m	997	kg/m^3
Electric constant	ε_0	8.85E+12	F/m
Medium conductivity	σ_m	0.015	S/m
Medium relative permittivity	ε_m	80	ε_0
Particle conductivity	σ_{mem}	0.001	S/m
Particle relative permittivity	ε_{mem}	2.5	ε_0
Radius of particle	r	7 e-6	m

REFERENCES:

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SUPPORTING FIGURES AND FIGURE LEGENDS

FIGURE S1: Amount of LDH released for SC23 HuNSPCs in growth medium over 6 hours. Low levels of LDH release, 2.8 ± 1.3 % and 7.3 ± 1.4 %, were observed for NSPCs in their growth medium for 2 and 6 hours, respectively. Condition 0% is baseline (cells in DEP buffer) and 100% represents the LDH released from HuNSPCS completely lysed using Triton X-100.

FIGURE S2: Cell survival assays performed on SC23 HuNSPCs after DEP exposure. Cell survival assays were performed on SC23 HuNSPCs using trypan blue exclusion, LDH release, and MTT reduction after short-term DEP exposure (*A, C,* and *E*) or long-term DEP exposure (*B, D,* and *F*). Data are represented as mean ± SE. Asterisks (*) and double asterisks (**) denote values that were determined to be significantly different from controls (*, *p*-value < 0.05 and **, *p*-value <0.01).

FIGURE S3: Cell survival assays performed on mouse NSPCs after DEP exposure. Cell survival assays were performed on E12.5 mouse NSPCs using trypan blue exclusion, LDH release, and MTT reduction after short-term DEP exposure (*A, C,* and *E*) or long-term DEP exposure (*B, D,* and *F*). Data were represented as mean ± SE. Asterisks (*) and double asterisks (**) denote values that were determined to be significantly different from controls (*, *p*-value < 0.05 and **, *p*-value <0.01).

FIGURE S4: Cell cycle kinetics analysis performed on SC23 HuNSPCs after short-term DEP exposure. SC23 HuNSPCs were exposed to DEP for 1 minute and cell cycle kinetics analysis was performed for 0, 0.5, 1, and 2 cycling times. There was no significant influence of DEP on the cells. Data represented as mean ± SE.

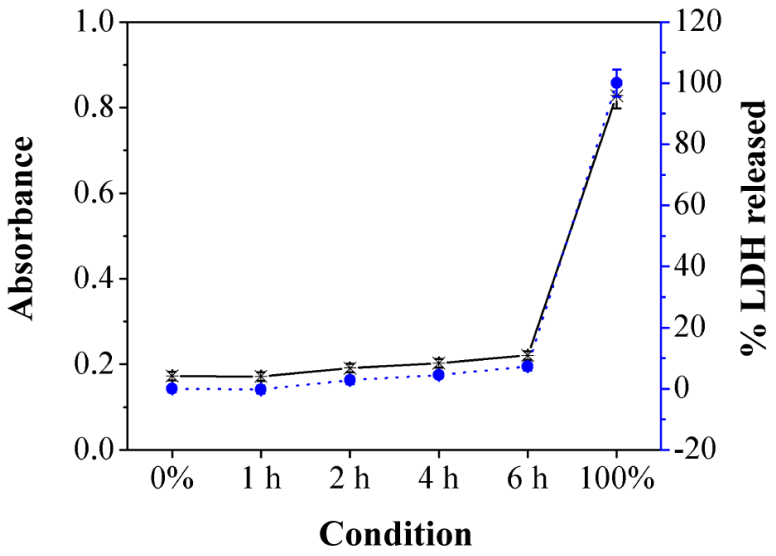

FIGURE S1

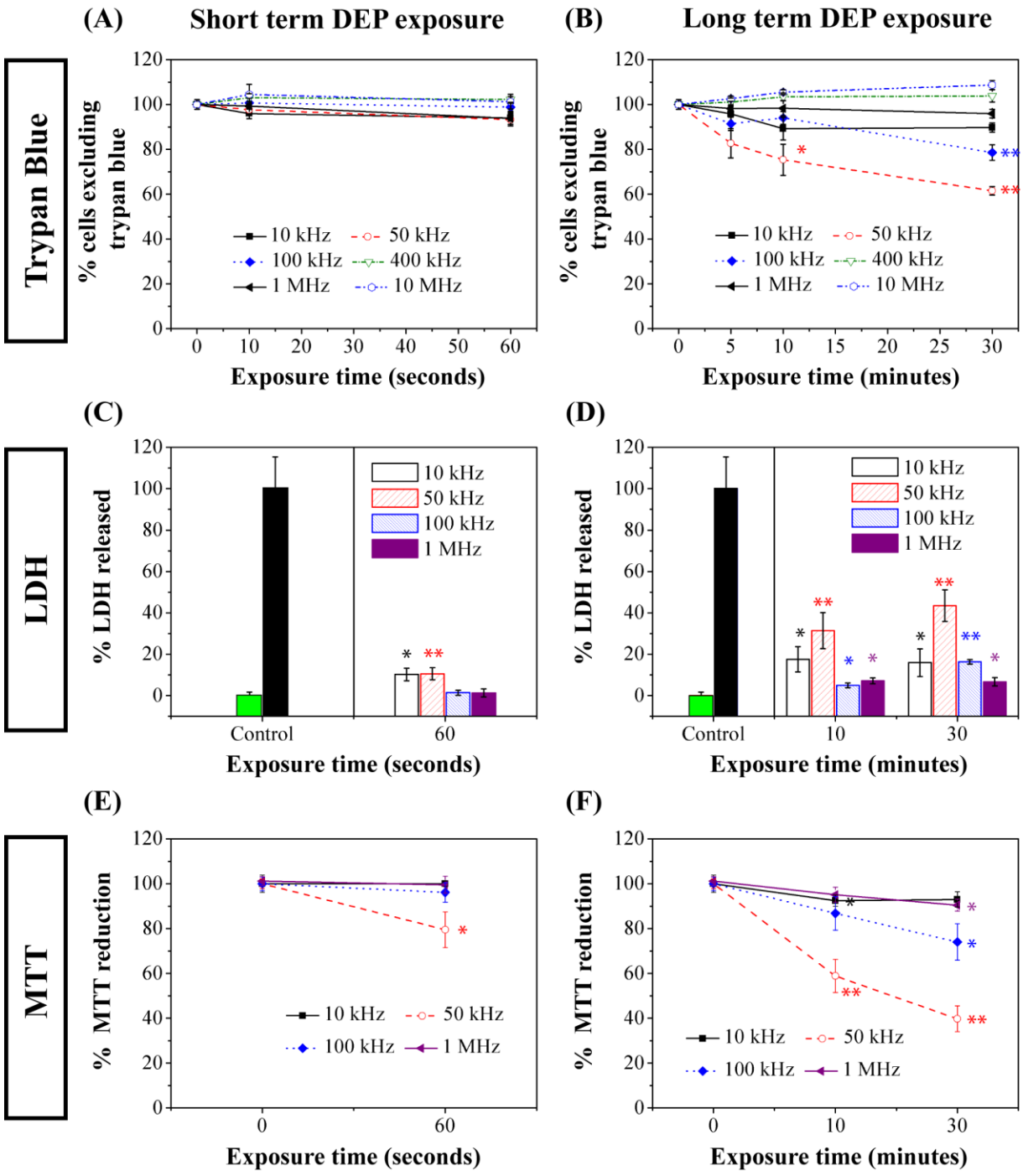

FIGURE S2

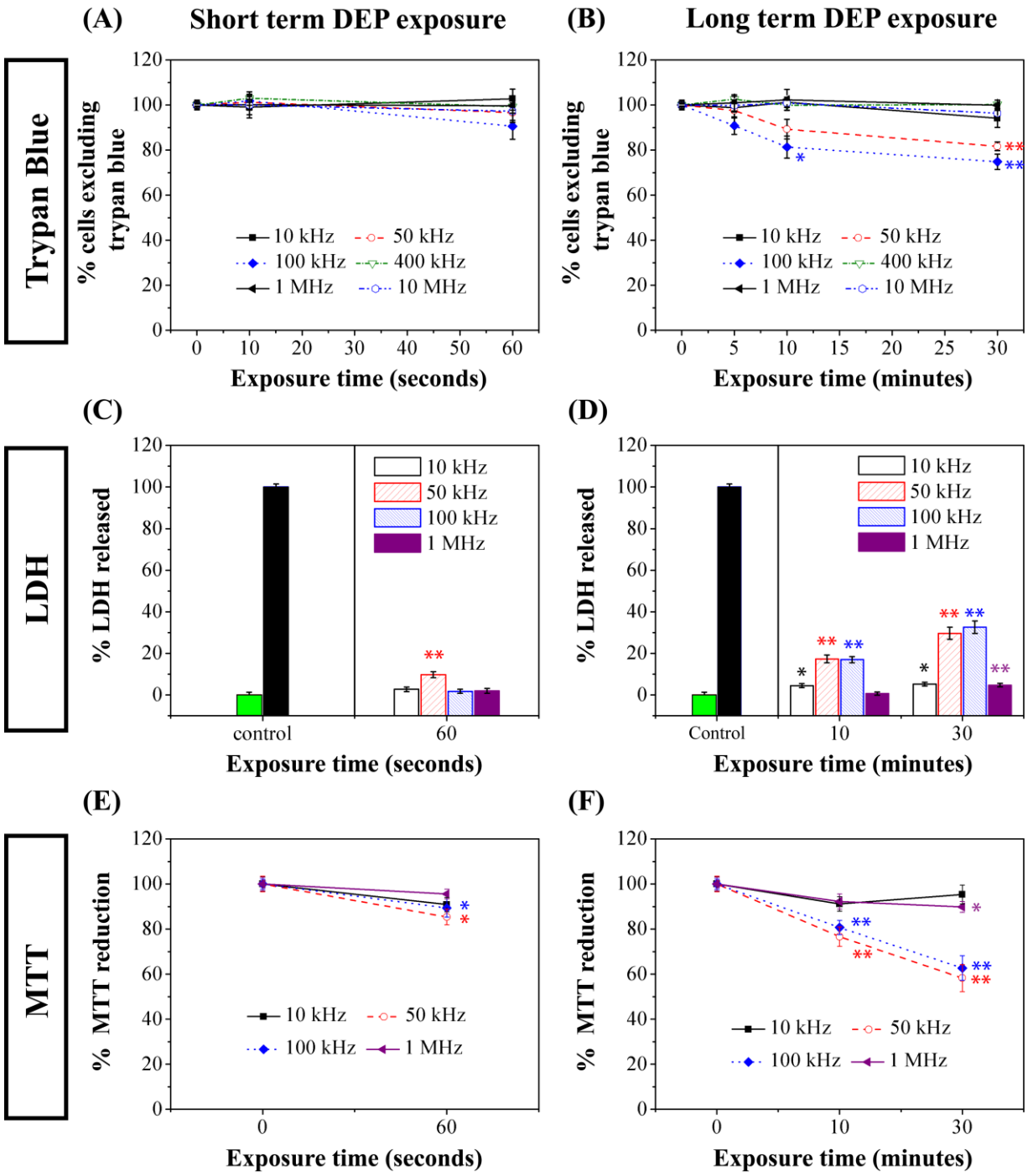

FIGURE S3

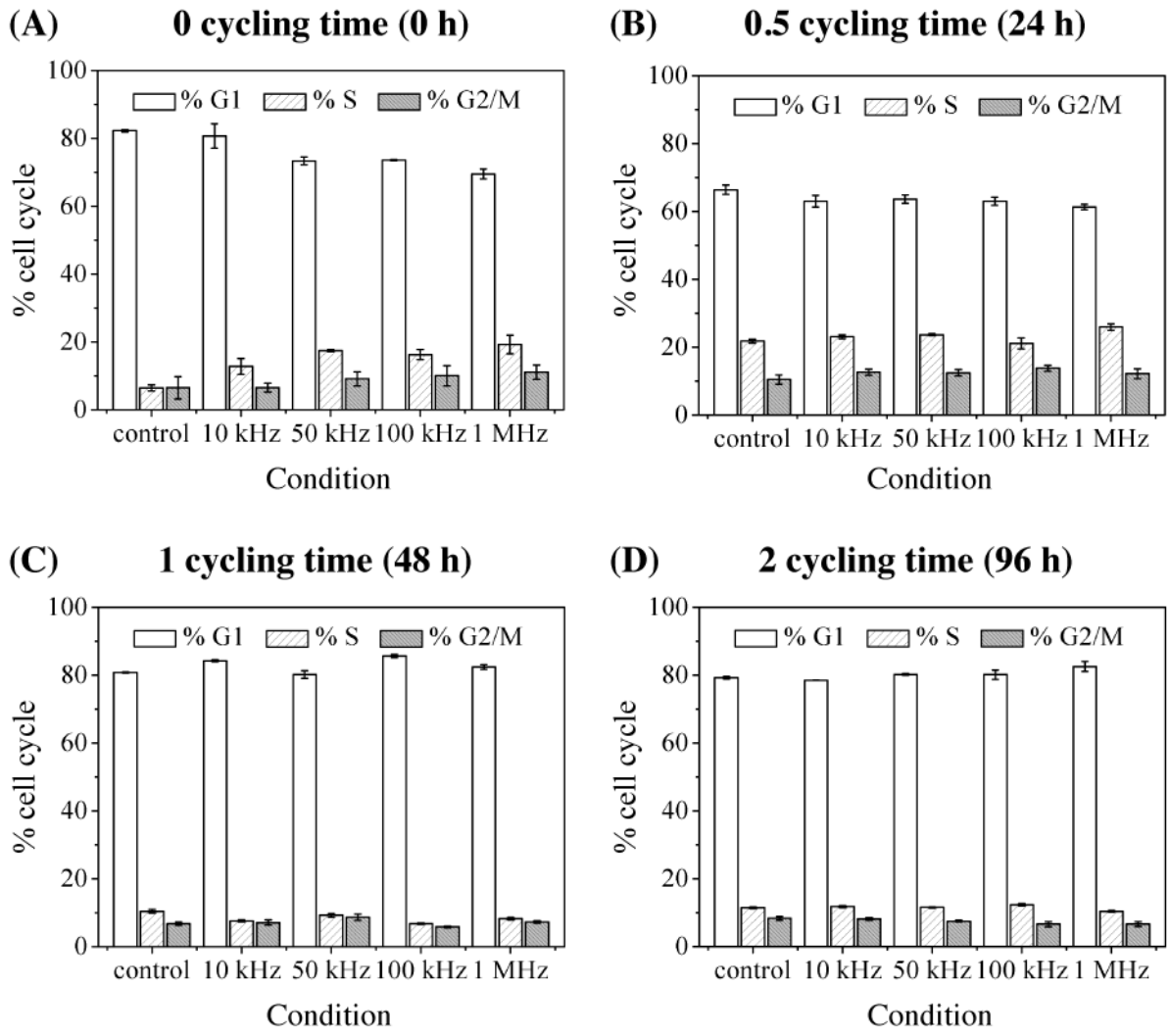

FIGURE S4