Supporting Information

Ultralong-recovery-time nanosecond electroporation system enabled by

orientational-disordering processes

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Table S1: Lipid composition of 1600-lipid bilayer system simulated.

	Inner leaflet	Outer leaflet
POPC	100	216
POPS	200	144
POPE	336	68
CHOL	136	136
PSM	28	236

Table S2: Statistical significance of the data in Fig. 3c, d.

		Time (min)						
		0	5	10	15	60	120	360
Compared to 0 pulse at t = 0, 5, 10, 15, 60, 120 and 360 min (control)	10 pulses		***		**	**		
	50 pulses		****	**	*	***	****	*
	100 pulses		***	***	****	***	****	*
	150 pulses		****	*	****	***	**	
	200 pulses		***	**	**	****	**	*

Table S3: Comparison of electrical parameters and recovery times of the nanosecond few-volt electroporation systems with that of state-of-the-art electroporation systems.

Ref. number	Paper	Cell type	Cell population	Pulse number	Pulse width	Gap distance	Field strength	Time of recovery
1	*Pharm.*, **12(5)**, 422 (2020)	CHO-K1 cells	65×10^3	10	200 ns	1 mm	$10 - 18$ kV/cm	10 min
2	*Bioelectrochemistry*, **140**, 107798 (2021)	CHO-K1 cells	240×10^3	1, 25, 100	200 ns, 400 ns, 550 ns	4 mm	$1.1 - 13.2$ kV/cm	~24 min
3	*J. Photochem. Photobiol. B Biol.*, **213**, 112066 (2020)	Sp2/0 cells	10×10^3	1–8, 250	100 ns to 1 ms	1 mm	$1 - 2.5$ kV/cm	60 min
4	*IEEE Trans. Biomed. Eng.*, **66(7)**, 2010–2021 (2019)	WB-F344 cell	$4 \times 10^3 - 8 \times 10^3$	8. 20, 60	100 ns	350 μm	~942 V/cm	210 min
	This Work	**PANC-1 cells**	**5×10^3**	**10, 50, 100, 150, 200 and 500 pulses**	**100 ns**	**0.1 mm**	**500 V/cm**	**720 min**

Table S4. Comparison of number of pulses utilized and cell viability for the few-volt nanosecond electroporation system with that for current electroporation systems.

Ref. number	Paper	Number of pulses	Pulse width	Cell viability
1	*Sci. Rep.* **11**, 1–10 (2021)	25	200 ns	~50% – 60%
2	*Cell Biol. Int. **35**, 99–104 (2011)*	30	450 ns	3.12%
3	*Bioelectrochemistry* **140**, 107837 (2021)	40	300 ns	<10%
4	*Cancer Res. Treat. **18**, 1–10 (2019)*	170	300 ns	~10%
5	**This work**	**200**	**100 ns**	**~99%**

Table S5: Comparison of model size utilized in this simulation with that for existing simulations.

Ref. number	Paper	Pulse number	Number of lipids	Field strength (V/nm)	Type of lipids	Simulation type
1	*Biophys. J.* **88**, 4045–4053 (2005)	1	64, 256	0.5, 1	DMPC	Atomistic
2	*Bioelectrochemistry*, **110**, 1–12 (2016)	1	144	0.25, 0.45, 0.65	DOPS, DOPC	Atomistic
3	*Biochim. Biophys. Acta - Biomembr.,* **1838**, 902–909 (2014)	2	512	0.6	DPPC	Atomistic
4	**This work**	**2**	**800, 1600, 2000**	**0.25**	**POPC, POPE, POPS, CHL1, SM16**	**Atomistic**
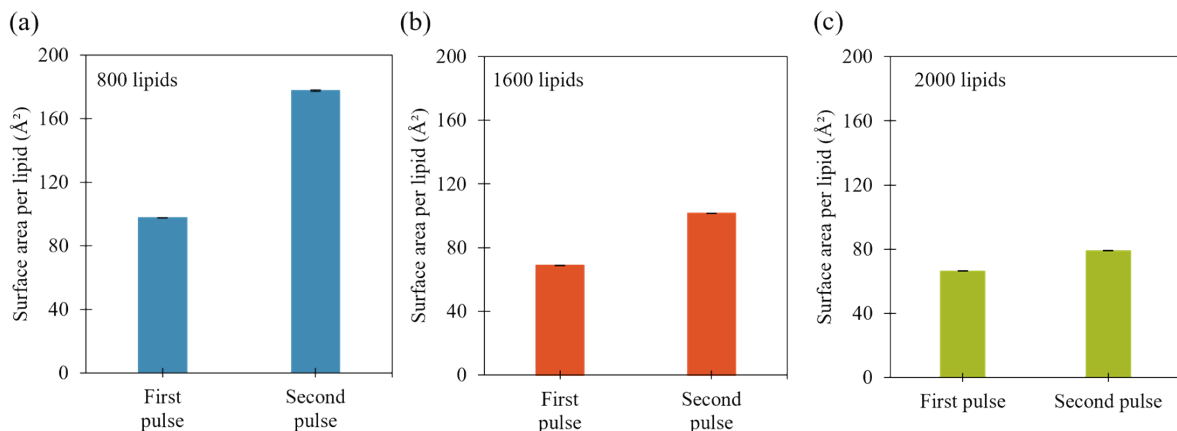
Figure S1. a–c) The surface area per lipid of the model with a) 800 lipids, b) 1600 lipids and c) 2000 lipids upon application of the first and second electric-field pulses. The error bars show the range of values obtained from simulations performed after applying the electric-field pulses (upon first pulse, 50.6–51.2 ns; thereupon second pulse, 51.8–52.4 ns).
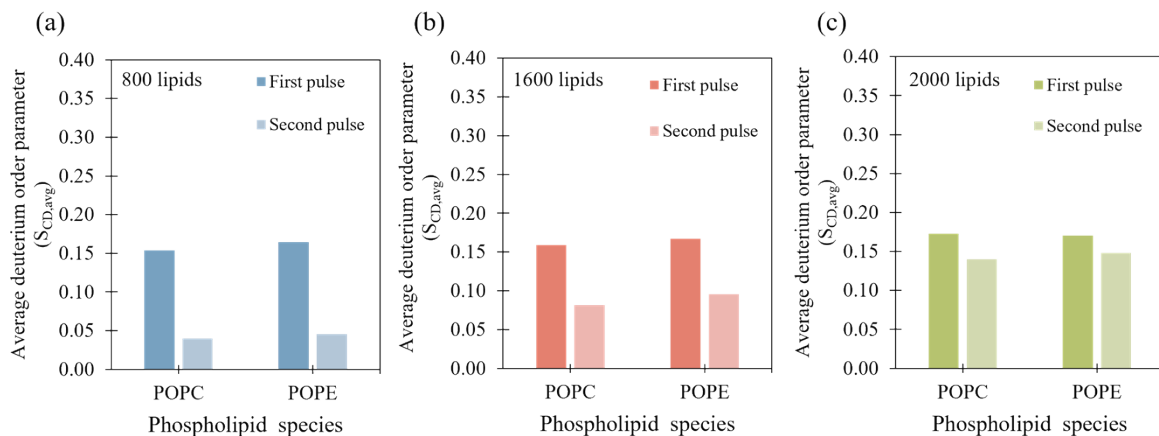

Figure S2. a–c) Average deuterium order parameters of the sn-1 chains of POPC and POPE in the lipid-bilayer model with a) 800 lipids, b) 1600 lipids and c) 2000 lipids after injecting the first and second electric-field pulses (upon 51.2 ns and 52.4 ns of simulations). The average deuterium order parameter is calculated via

$$S_{CD, \, avg} = \frac{\sum\limits_{i=1}^{N} S_{CD}}{N} \tag{1}$$

where $S_{CD,i}$ is the deuterium order parameter for a carbon atom, i is the index, 1 is the initial value of i and N is the final value of i ($N = 14$).
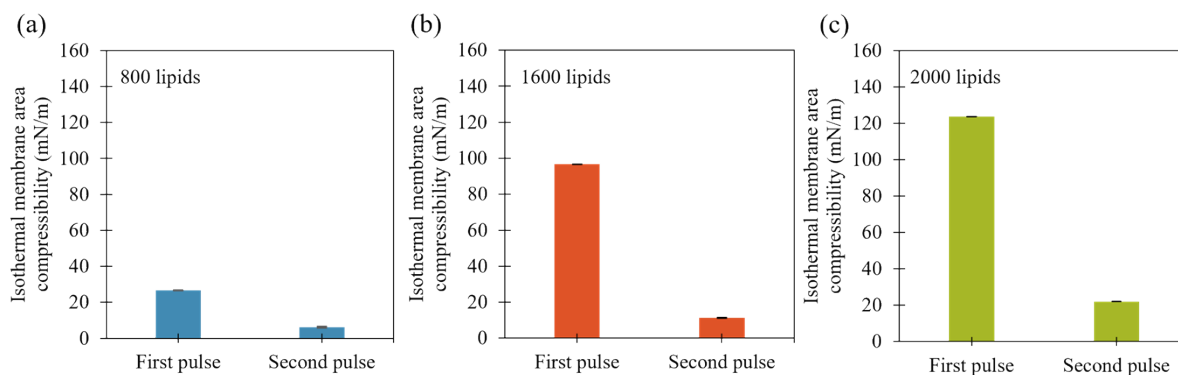
Figure S3. a–c) Isothermal membrane-area compressibility of the lipid bilayer model with a) 800 lipids, b) 1600 lipids and c) 2000 lipids upon applying the first and second electrical pulses. The error bars show the range of values obtained from simulations performed upon the application of electric-field pulses (after first pulse, 50.6–51.2 ns; following second pulse, 51.8–52.4 ns).

For the bilayer model with 1600 lipids, when the number of pulses increases, the models disclose a larger surface area per lipid (Fig. S1b). Additionally, the model demonstrates a smaller deuterium order parameter for POPC and POPE with an increase in number of pulses (Fig. S2b). Furthermore, an increase in the number of pulses results in the model showing a smaller isothermal membrane area compressibility (Fig. S3b). For the bilayer models with 800 and 2000 lipids, similar changes are observed after the application of first and second electric-field pulses (albeit with different magnitudes of changes) (Supporting Fig. S1–S3), which indicates that the model size does not affect trend-of-changes.
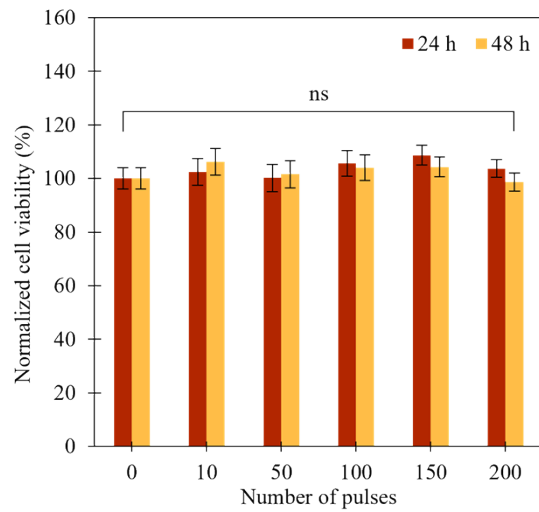
Figure S4: Normalized cell viability of the cells after electroporation at 24 h and 48 h. The error bars show the range of values obtained from experiments performed on 6 different systems.
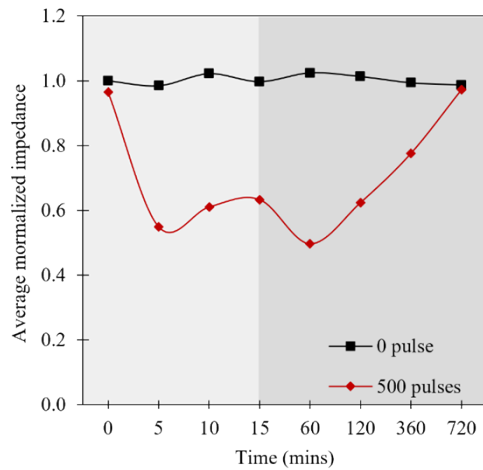

Figure S5. Time evolution of average normalized impedance of the system upon application of different number of pulses (0 and 500 pulses). The average normalized impedance is calculated using the ratio of sum of normalized impedance for n different systems to the number of systems n ($n = 6$).
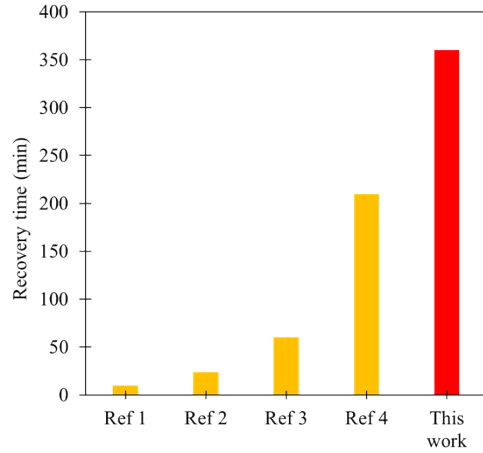
Figure S6: Comparison of recovery time of nanosecond electroporation systems. The information of the references can be found in Table S3.
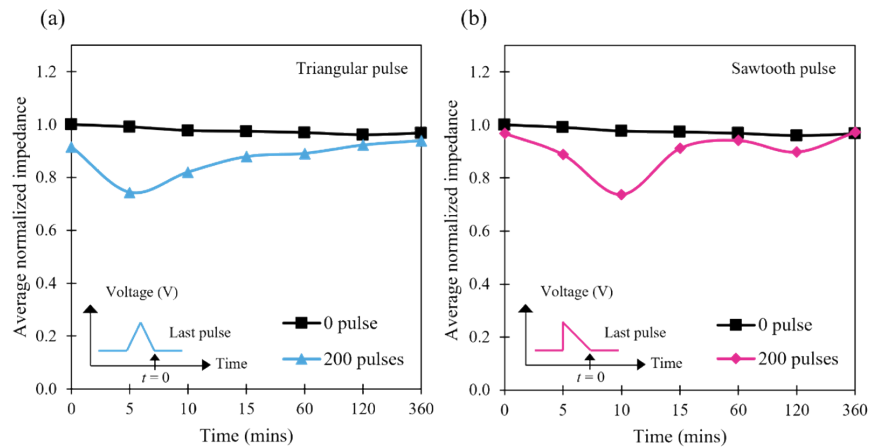

Figure S7. a, b) Time evolution of average normalized impedance of the system upon application of different number of pulses for a) triangular-based pulse and b) sawtooth-based pulse. The average normalized impedance is computed by the ratio of sum of normalized impedance for n different systems to the number of systems n ($n = 6$).

For the square-based pulse, when a strong pulse is injected to the cells, the pulse can result in an electric field/ bias voltage above the transmembrane voltage for pore formation.[8, 9] The duration of pulse amplitude beyond the transmembrane voltage is sufficient to form a large population of pores to achieve a strong membrane permeabilization (for the 0–360 min time range, the cells after the application of electric field pulses disclose a small average normalize impedance) (Fig. 3c). For the triangular-/ sawtooth-based pulse, it is possible that the duration of pulse

amplitude exceeding the transmembrane voltage is *not long enough* to form a large pore population, resulting in a weak membrane permeabilization (the cells upon the injection of electric-field pulses exhibit a large average normalized impedance in the 0–360 min time range).
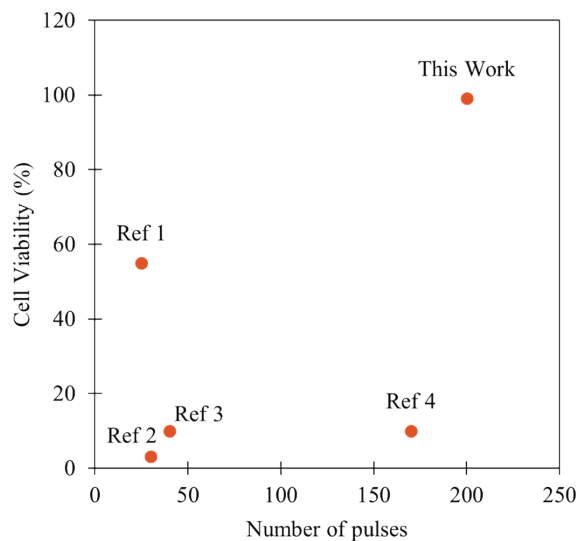
Figure S8. Comparison of the number of pulses utilized and cell viability for electroporation systems. The information of the references can be found in Table S4.
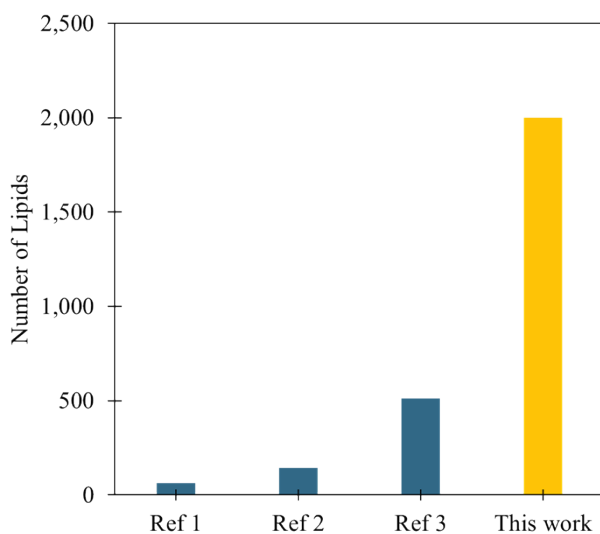

Figure S9. Comparison of the model size utilized for MD simulations in the electroporation of lipid bilayers. The information of the references can be found in Table S5.