# **ELECTRONIC SUPPLEMENTARY INFORMATION**

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# Title: Microfluidic Electro-Sonoporation: A Multi-Modal Cell Poration Methodology through Simultaneous Application of Electric Field and Ultrasonic Wave

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## **Electric Field and Acoustic Field Simulation**

To see whether uniform electric field and acoustic field are applied to the cells flowing through the microchannels, finite element method (FEM) simulation was conducted. Electric potential, electric field and acoustic field were simulated and represented by the color gradient and arrow volume in Supplementary Fig. S1. Electrical conductivity of  $1.44 \times 10^7$  S/m and  $5.5 \times 10^{-6}$  S/m, speed of sound of 4900 m/s and 345 m/s, and density of 8908 kg/m<sup>3</sup> and 1000 kg/m<sup>3</sup> were selected for nickel and water, respectively. The electric potential inside the channel was shown as a gradient distribution because one electrode was set at 10 V and the other electrode was grounded. As can be seen from the Supplementary Fig. S1, both the electric field and acoustic field inside the microfluidic channel were uniform throughout the channel. Thus it was expected that cells flowing through the microfluidic channel will be exposed to uniform electroporation and sonoporation effect.



Supplementary Fig. S1 FEM simulation result of (a) the electric potential, (b) the electric field, and (c) the acoustic pressure field.

#### Comparison of Electroporation, Sonoporation, and Electro-sonoporation Results

A detailed summary of the experimental results and comparison of these results to other previously published results are presented here. Supplementary Table 1 and Table 2 summarize both poration efficiency and cell viability results of the five repeats we have conducted.

As shown in Supplementary Table 1, microelectro-sonoporation shows average improvement of 17.9% over microelectroporation and 10.8% improvement over microsonoporation in poration efficiency (Student's T-test p < 0.05). When comparing the average improvements in cell viability as shown in Supplementary Table 2, microelectro-sonoporation shows average improvement of 8.0% over microelectroporation (Student's T-test p < 0.05) and no improvement over microsonoporation.

Experiments	Experiments Efficiency (%)			Improvement of E + S <sup>c</sup> over E <sup>a</sup> (%)	Improvement of E + S <sup>c</sup> over S <sup>b</sup>	
	E <sup>a</sup>	S <sup>b</sup>	$\mathbf{E} + \mathbf{S}^{c}$		(%)	
Experiment 1	72.2	87.8	94.7	22.5	6.9	
Experiment 2	75.9	89.3	99.2	22.3	9.9	
Experiment 3	81.4	86	95.2	13.8	9.2	
Experiment 4	81.6	80.3	93.7	12.1	13.4	
Experiment 5	77.8	81	95.4	17.6	14.4	
Average ± STD	77 <b>.8</b> ± 4.0	<i>84.9</i> ± <i>4.0</i>	95.6 ± 2.1	17.9 ± 5.0	<i>10.8</i> ± <i>3.1</i>	

Supplementary Table 1 Summary of Poration Efficiency

<sup>a</sup> E: microelectroporation.

<sup>b</sup> S: microsonoporation.

<sup>c</sup> E + S: microelectro-sonoporation.

Experiments	Viability (%)			Improvement of E + S <sup>c</sup> over E <sup>a</sup> (%)	Improvement of $\mathbf{E} + \mathbf{S}^{c}$ over $\mathbf{S}^{b}$ (%)	
	E <sup>a</sup>	S <sup>b</sup>	$\mathbf{E} + \mathbf{S}^{c}$	1		
Experiment 1	83.9	98.9	96.7	12.8	-2.2	
Experiment 2	86.8	99.8	100	13.2	0.2	
Experiment 3	94.9	94.5	98.1	3.2	3.6	
Experiment 4	92.1	97.1	95.6	3.5	-1.5	
Experiment 5	88.9	95.2	96.2	7.3	1.0	
Average $\pm$ STD	89.3 ± 4.3	<i>97.1</i> ± <i>2.3</i>	97.3 ± 1.8	<i>8</i> ± <i>4</i> . <i>8</i>	0.2 ± 2.3	

#### Supplementary Table 2 Summary of Cell Viability

<sup>a</sup> E: microelectroporation.

<sup>b</sup> S: microsonoporation.

<sup>*c*</sup> E + S: microelectro-sonoporation.

A summary of the transfection efficiency and cell viability for HeLa cell electroporation is shown in Table 1 of the manuscript. Our microelectroporation result shows an average efficiency of 77.8% with viability of 89.3%. An obvious advantage of microelectroporation is evident compared to conventional electroporation in both transfection efficiencies and cell viabilities, both from our result and other previously reported results. The typical improvement in efficiency between the conventional and microchip methods is almost two-fold. Kim *et al.* provided a comparison of macro- and micro-electroporation for HeLa cells exposed to similar electric field strengths,<sup>1</sup> in which conventional electroporation showed an approximate transfection efficiency of 40% with cell viability of 67%, whereas microelectroporation results to other microelectroporation reports for HeLa cells, our data are similar or slightly lower in terms of transfection efficiency (77.8% vs. 75 - 85%), however outperforms others in cell viability (89.3% vs. 77 - 80%). This might have been attributed by the limited electric field exposure time due to the continuous flow configuration in our device.

It is important to note that a direct comparison across all data in the table may be difficult, as different methods are used for

assessing transfection efficiency. Namely, a distinction must be made between using fluorescent dye and genetic expression (primarily with EGFP) as indicators of transfection. In Table 1, Glahder *et al.*<sup>2</sup> (conventional electroporation) and Kim *et al.*<sup>1</sup> (microelectroporation) used EGFP while the reported data shown for Rodamporn *et al.*<sup>3</sup> (conventional), He *et al.*<sup>4</sup> (microelectroporation), and our work (microelectroporation) all used PI as the marker of permeability. Thus, by comparing the results only from the latter group using PI, a significant improvement from macro-<sup>3</sup> to microelectroporation<sup>4</sup> is still evident and our microelectroporation results are comparable to those reported by He *et al.*<sup>4</sup> but with much higher cell viability.

A summary of the transfection efficiency and cell viability data for HeLa cell sonoporation is shown in Table 2 of the manuscript. Again, it should be noted that a direct comparison of all results may be difficult due to the different methods used for assessing transfection efficiency. In Table 2, all results shown, with the exception of our data for both sonoporation and electro-sonoporation, used some sort of genetic material for the transfection assay. However, Lai et al.<sup>5</sup> used DNA-FITC molecules, which merely assess uptake (similar to dyes), not genetic expression (all others<sup>6-8</sup> used EGFP). When comparing our microsonoporation results with conventional sonoporation (Lai et al.<sup>5</sup>), the improvements in both efficiency and viability are substantial, approximately two-fold in both cases (87.9% vs. 35%, 97.1% vs. 45%, respectively). In fact, our results, along with those reported by Rodamporn et al.,<sup>8</sup> greatly outperform all others in terms of transfection efficiency. When examining the experimental protocol, it seems that the basis for this large improvement with microsonoporation is similar to the reasons for improvements between electroporation and microelectroporation. Rodamporn et al. demonstrated UCA-free sonoporation in a microfluidic chamber with a single piezoelectric ceramic, which utilized a microfluidic platform to greatly reduce transducer-toreflector spacing and thus generate a highly uniform acoustic field at resonance frequency;<sup>8</sup> similarly, we use a microfluidic scheme to manipulate a suspension of cells into a uniform layer, so that all cells are in relatively the same acoustic plane. The amount of exposure of a population of cells in a microfluidic channel to the applied acoustic field is highly uniform when compared to cells exposed in cuvette-type suspensions. Thus, we term this configuration as microsonoporation. Similarly, Kinoshita et al. compared conventional sonoporation results for suspensions of cells versus cell monolayers.<sup>9</sup> Using Calcein AM as a marker of cell membrane integrity in C166 cells monolayers of cells showed an increase of approximately 10% in efficiency and a 50% improvement in cell viability compared with those treated in suspensions. When comparing our microsonoporation results to HeLa cell microsonoporation result from Rodamporn et al.<sup>8</sup>, our data shows remarkable improvement in both transfection efficiency (87.9% vs. 68.9%) and cell viability (97.1% vs. 77%).

The combined simultaneous microelectro-sonoporation result from our device clearly shows that combining two modalities results in higher efficiency while maintaining or outperforming the already high viability of microscale electroporation or sonoporation. Comparing this result to previously reported HeLa cell microelectroporation results, the efficiency was similar (77.8% vs. 75 - 85%) but the viability was much higher (89.3% vs. 77 - 80%). Furthermore, when comparing our microelectrosonoporation method to any of the various reports on sonoporation, it greatly excels in both transfection efficiency and cell viability.

# **Cell Poration Criteria**

The criteria for determining successful cell poration are described in the main text. The criteria in determining a cell to be fluorescent are 1) at least 50% of the cell area needs to show fluorescence (see Supplementary Fig. S2(a) where the marked cell is NOT counted as fluorescence) and 2) any cell that is showing significantly weaker fluorescence (less than 40% mean intensity) is NOT counted as fluorescence. An example is shown below to further clarify these criteria.



Supplementary Fig. S2 Criteria for determining successful poration of cells. (a) The cell marked with an arrow shows green fluorescence in less than 50% of its cell area, therefore not counted as viable. (b) The cells marked with arrows show weak red fluorescence (less than 40% of mean intensity of other porated cells), therefore are not counted as successfully porated, however are viable as they show strong green fluorescence.

## **Additional Cell Images after Poration**

Additional images of cells after microelectroporation, microsonoporation, and microelectro-sonoporation are shown in Supplementary Fig. S3. For each condition, three randomly picked example cell images are shown in both brightfield and fluorescence (red and green). Left three sets show cells with 10X magnification and the right two image sets show cells with 20X magnification. As described in the main text, cells that exhibit both red and green fluorescent are deemed to be successfully porated, or transfected. Cells that are only fluorescing green indicate that these cells are alive, but without successful poration, whereas cells that are only fluorescing red indicate that these cells are either dead from the beginning or died during the poration process, also some cell debris can be seen as red which is not counted.

Brightfield	Brightfield Calcein AM		Calcein AM	Propidium						
(a) Microelectroporation										
1			4 4 4	•						
. 11										
(b) Microsonopor	ation									
				· · ·						
<b>II</b> .										
III			e si se							
(c) Microelectro-sonoporation										
•			· · · ·							
1										
		20 <u>0 µm</u>		10 <u>0 µ</u> m						

**Supplementary Fig. S3** Additional images of cells treated with (a) microelectroporation, (b) microsonoporation, and (c) microelectrosonoporation, respectively. For each condition, three randomly selected brightfield and fluorescent images are shown in 10X (left) and 20X (right) magnification. Scale bar =  $200 \,\mu\text{m}$  in 10X images, and 100  $\mu\text{m}$  in 20X images.

### References

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