

# ON-CHIP SEQUENTIAL MOLECULE DELIVERY INTO ISOLATED CELLS USING VORTEX ASSISTED ELECTROPORATION

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## ABSTRACT

We demonstrated a single-cell level microscale electroporation system, allowing sequential delivery of various molecules into pre-selected identical populations of target cells. Inherently membrane-impermeant molecules can be transferred uniformly across entire cytosol with a precisely controlled amount. Additionally, the system allows real-time monitoring of on-chip biomolecule delivery processes without the need for additional target-cell purifications pre- or post-electroporation steps, minimizing costly reagent usage and sample loss. The system's high sample viability and electroporation efficiency with minimized sample-loss suggest that the current system has great potential to expand research fields that on-chip electroporation techniques can be used.

## KEYWORDS

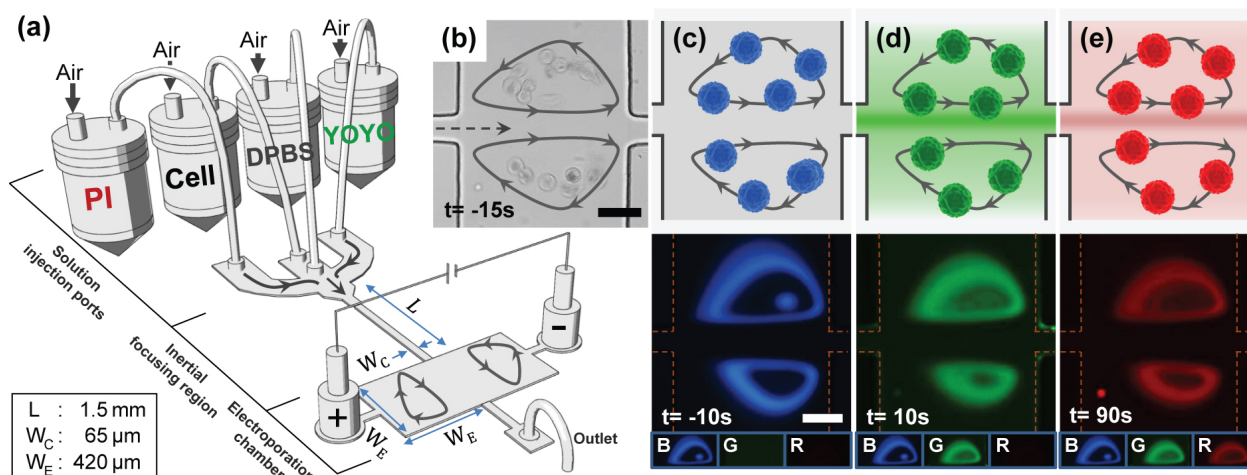
Molecule delivery, Electroporation, On-chip sample preparation, Cell purification.

## INTRODUCTION

The ability to introduce foreign molecules into biological components, such as living mammalian cells and human tissues, has significant implications for biological research and medical applications [1]. Various chemical and physical methods have been developed in order to manipulate and/or alter cellular functions by delivering molecular probes into living cells. Among these techniques, electroporation is an effective technique that permits direct labeling of intracellular components or regulating cell function by transiently creating pores through which molecular probes can be introduced. [1, 2]. Its ability to physically inject molecular probes into cells eliminates need for potentially cell-damaging chemical reagents or viruses. However, conventional electroporation techniques utilizing cuvettes or micro-capillaries rely on bulk stochastic molecule delivery processes, prohibiting sequential delivery of multiple molecules into identical cell populations with precisely controlled dosage [3, 4]. In addition, the conventional electroporation methods cannot be easily integrated with real-time monitoring techniques for direct visualization, useful for studying uptake mechanism of probes [4]. In order to address these limitations, we have developed a microfluidic vortex-assisted electroporation system, allowing the sequential delivery of multiple molecules into pre-selected identical population of target cells at the single-cell level.

## EXPERIMENTAL METHODS

The sequential vortex-assisted microscale electroporation system consists of sample and solution injection ports, the inertial focusing region, electroporation chambers, and an outlet (Figure 1). The inlet region has multiple injection ports assigned for solutions containing (i) biological samples to be electroporated (sample solution), (ii) biomolecules to be delivered to electroporated biological samples (molecule solutions), and (iii) cell growth media or DPBS that washes the entire device in-between step and in which processed cells are resuspended (washing solution). The sample solution, containing heterogeneous cell population of metastatic breast cancer cell line (MDA-MB-231) or leukemia cell line (K562), is injected into the device using a custom-build pressure system at operating pressure of 40 psi, equivalent to the flow rate of 400  $\mu$ L/min. In the inertial focusing region, flowing cells

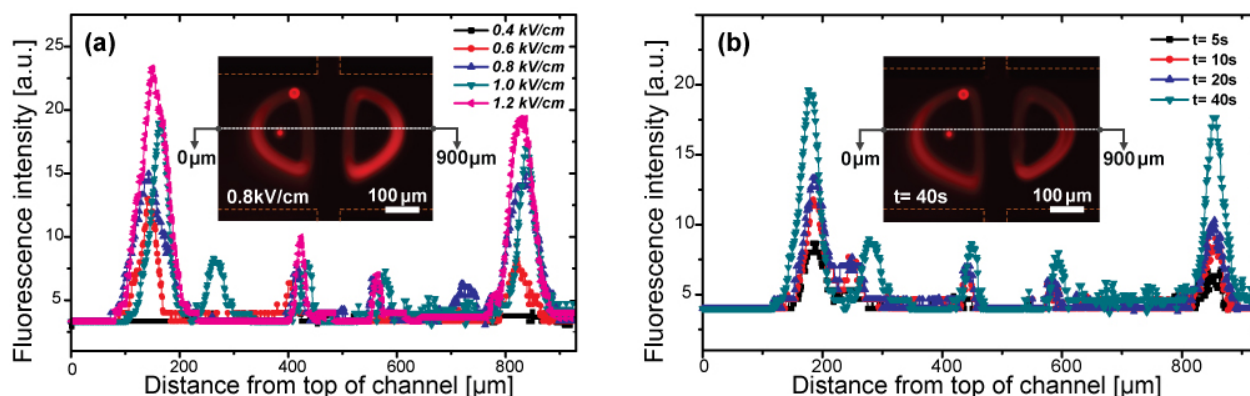


**Figure 1.** Schematics of On-Chip Sequential Electroporation System and electroporation of MDA-MB-231. (a) The system consists of four injection ports, inertial focusing region, electroporation chambers with electrodes and an outlet. (b and c) Calcein AM (B) stained target cells are isolated into the electroporation chamber. (d and e) Shortly after electroporation, molecular probes (G: YoYo<sup>®</sup>-1 and R: PI) are sequentially injected through the device and uptaken by trapped cells. Scale bars are 100  $\mu$ m.

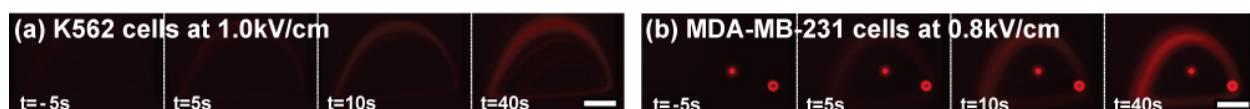
are precisely positioned at distinct lateral equilibrium positions depending on their biophysical properties [5]. Among inertially focused flowing cells, only cells with diameter greater than a trapping threshold ( $D_{cr} \approx 12\mu\text{m}$ ) are isolated and trapped in electroporation chambers [6, 7]. Once target cells are trapped in those chambers, the sample solution is rapidly exchanged with the washing solution in order to remove unwanted small cells from the entire device without disturbing orbits that trapped cells created. Trapped cells are electroporated by applying short pulsations of high electric field and, promptly after short pulsations, electroporated cells are exposed to molecule solutions one by one using the rapid solution exchange scheme [6]. After controlled amount of molecular dosage has been injected through the device, the electroporated sample are resuspended into the washing solution and released from the device for downstream analysis by simply lowering the operational pressure to 5 psi. A custom-built, computer-assisted pressure control setup is used to control flow through the system in order to stably maintain identical cell populations in the designated chambers throughout the course of electroporation and multiple solutions exchange steps. Inherently membrane-impermeable molecules, including two DNA intercalating agents (Propidium iodide (PI) and YoYo<sup>®</sup>-1) and an enhanced green fluorescent protein (EGFP) plasmid, were successfully transferred into selected cell populations. Finally, the efficiency of electroporation and viability of processed mammalian cells were evaluated by systematically varying (i) solution injection time and (ii) electroporation voltages.

## RESULTS AND DISCUSSION

Real-time monitoring of fluorescent signals exhibited that (i) there exists a distinct electric field threshold that molecule-uptake is initiated for each cell line tested and (ii) the amount of transferred molecules is gradually increased with increasing electric field strengths and molecular doses (Figure 2 and 3).

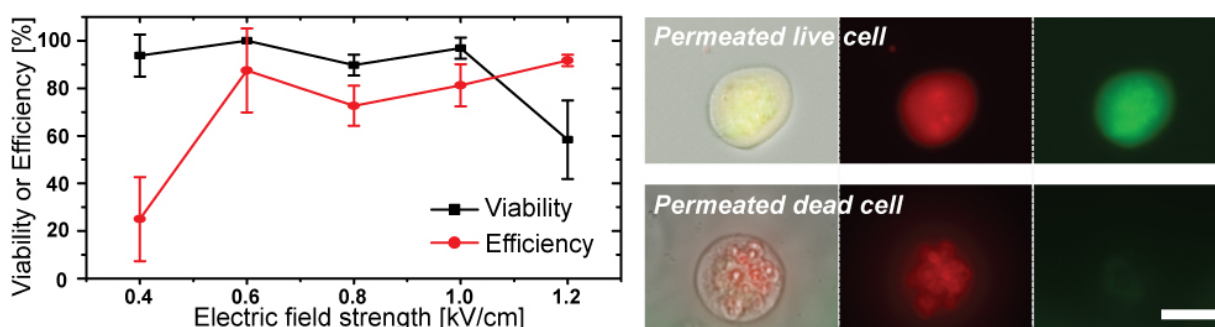


**Figure 2.** The amount of transferred PI molecules into MDA-MB-231 cells increased with (a) increasing electric field strength and (b) increasing solution injection time at  $E=0.8\text{kV/cm}$ . The electric pulses was applied at  $t=0$ .



**Figure 3.** Sequential fluorescent images illustrating gradual increase of fluorescent signals of electroporated K562 and MDA-MB-231 cells. The tested uptake material was PI and scale bar is  $100\mu\text{m}$ .

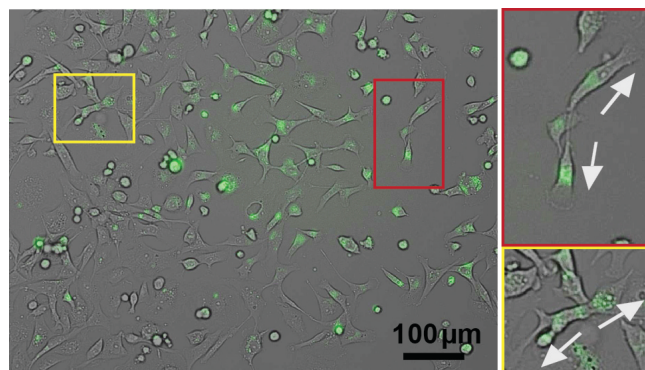
The viability and electroporation efficiency tests were conducted on electroporated cells collected off-chip revealed that the optimum electric field strengths for K562 and MDA-MB-231 cells were found to be approximately electric field strength,  $E = 1.0\text{kV/cm}$  and  $0.8\text{kV/cm}$ , respectively, with viability greater than 83% and electroporation efficiency of 70%. In addition, the distribution of transferred molecules was uniform throughout the entire cytosol, indicating uniform permeabilization that potentially related to reduced mortality (Figure 4).



**Figure 4.** Viability and efficiency of electroporated MDA-MB-231 cells ( $n=4$ ). The optimum electric field strengths for MDA-MB-231 were found to range between 0.6 and 1.0 kV/cm with the viability greater than 83% with the electroporation efficiency of 70%. Scale bar is  $10\mu\text{m}$ .

Moreover, a single electroporation step was found to be sufficient enough to sequentially deliver two distinct membrane-impermeable molecules (PI and YoYo<sup>®</sup>-1) into pre-isolated MDA-MB-231 cells through using sequential solution exchange processes (Figure 1 c–e).

Finally, MDA-MB-231 cells are transfected with an EGFP plasmid labeling endoplasmic reticulum (Figure 5a) using the optimum electroporation condition of the system ( $E = 1.0\text{ kV/cm}$  and plasmid concentration of  $50\text{ }\mu\text{g/ml}$ ). Transfected cells exhibited strong and localized fluorescence signals around nuclear region after culturing cells off-chip for one week.



**Figure 5.** Images of EGFP transfected MDA-MB-231 cells cultured for a week. Microscopic image of MDA-MB-231 cells electroporated at  $1.0\text{ kV/cm}$  for 20ms (overlay of bright field and green fluorescent images). (right) Magnified images showing divisions of transfected cells. Image contrast is enhanced by adjusting look-up table.

## CONCLUSION

The proposed technique allows cost-effective, single-cell level, on-chip molecular probe delivery without the need for additional target-cell purifications pre- or post-electroporation steps (i.e., laborious sample preparations or time-consuming centrifugation). The amount of transferred molecules can be precisely and individually controlled for various molecules and can be delivered into the identical target cell populations by a single electroporation process. The system would offer numerous benefits, including real-time monitoring of intracellular transferred molecule amounts, reduction in costly reagent usage and minimized electroporated sample loss. Moreover, the system's low operational current can eliminate viability-related problems associated with a high current in electroporation. The developed technique would have great potential to expedite the development of the cost-effective electrochemotherapy or the gene electrotransfer process, useful for the cancer therapy.

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