

SELECTIVE HIGH EFFICIENCY IN-FLOW ELECTROPORATION WITH FOCUSED ELECTRIC FIELDS IN A MICROSYSTEM

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Abstract

We present a new technique for delivery of macromolecules into biological cells in suspension by in-flow electroporation which achieves reproducible high delivery efficiency with excellent post-electroporation viability and enables delivery to selected cell types in mixtures. We detect cells via impedance change as they flow through a microfabricated *sense-porate aperture* and then electroporate them by creating a transient zone of focused high electric field by applying a low voltage pulse. We describe here the device principle and demonstrate the delivery of FITC-labelled 150kD Dextran molecules to large numbers of cells of a human squamous cell carcinoma line with very delivery efficiency (~98%) and very low cell mortality (~11%).

Keywords: Electroporation, Targeted delivery, High Efficiency, High Viability

1. Introduction

Electroporation is a method for delivering cell membrane impermeant charged or polar molecules such as DNA, RNA, proteins and drugs to cells by transiently breaching their membranes using high electric fields. In conventional 'plates-in-vial' electroporators, this usually needs high voltages (~kVs) and yields poor and irreproducible delivery efficiencies (~50-60%) and poor cell viabilities (~30-40%) due to excessive heating and lack of control on the field experienced by individual cells [1]. Furthermore, cell-specific targeted delivery is inconceivable in this method. Recent chip-based electroporators [2,3] have used visually assisted cell trapping at orifices or junctions which limits their usefulness as tools for practical biological research requiring large number of cells.

2. Principle

We perform in-flow electroporation of cells in suspension as they pass at a controlled rate through a tapered hole in silicon that we refer to as the *sense-porate aperture*. This is depicted in Figure 1. The small-signal ac impedance of the aperture is continuously monitored using electrodes placed across it. Cells are detected by a rise in this impedance as they approach the narrow end of the aperture. A pre-programmed impedance-based parameter is evaluated for each incoming cell and if it lies within a specified window, a voltage pulse is applied to the electrodes to create a high electric field zone at the narrow end of the aperture. As the cell passes through this zone, it undergoes electroporation and the suspended macromolecules are delivered to its cyto-

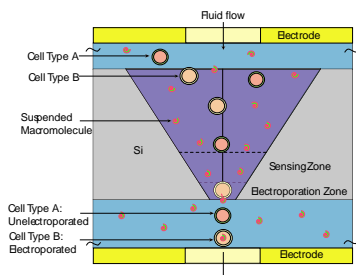


Figure 1. Principle of in-flow selective electroporation in a microfabricated *sense-porate aperture*. Cells moving through the tapered aperture are sensed and identified by their impedance and then electroporated in a selectively triggered controlled electric field zone.

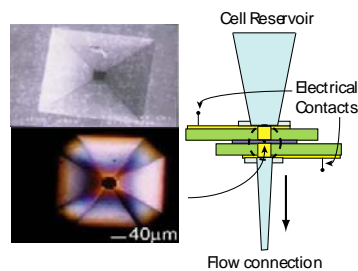


Figure 2. SEM and optical micrographs of the fabricated *sense-porate aperture* – a thermally oxidized anisotropically etched through hole in silicon. This is mounted between PCBs with gold-plated electrodes and plastic flow connectors.

-plasm. This impedance-signature-triggered transient generation of a small zone with adequately high electric field enables automated, continuous and controlled, in-flow electroporation of individual cells from a suspension containing a large number of cells without the need for any optical tagging and manual cell trapping.

3. Experimental Methods

The *sense-porate aperture* is an anisotropically etched, thermally oxidised through hole in silicon which was fabricated using standard microfabrication techniques as described in [4]. Figure 2 shows optical and SEM micrographs of the fabricated aperture which is designed to be $40\mu\text{m} \times 40\mu\text{m}$ at the narrow end. This is packaged in a flow-cell for electrical and flow connections. An electroporation setup with custom hardware and software [4] is then used to implement the scheme. The cell line used in the study is a human squamous carcinoma cell line (NT8e) which was grown using standard cell culture methods and harvested and finally suspended in the run buffer i.e. 1XPBS with 1% Bovine Serum Albumin (BSA) and kept on ice.

4. Results and Discussion

Impedance sizing of two particle sizing standards and the target cells was performed to calibrate the impedance sensing electronics. Voltage peak height distributions obtained for a mixture of $10\mu\text{m}$ and $20\mu\text{m}$ diameter particles and for the target NT8e cell population are shown in Figures 3 & 4.

The electroporation voltage was set to 8V and the cell suspension flow rate was set to 20nL/sec to achieve sufficient transmembrane potential ($\sim 0.2\text{-}1\text{V}$) and electroporation time ($\sim 1\text{ms}$) for this cell size. NT8e cells were electroporated in a suspension with a cell-membrane impermeant green fluorescent FITC-tagged dextran (MW=150kD). Cells kept in an identical suspension without electroporation were used as a control sample. Propidium iodide, a live-cell impermeant red fluorescent dye, was then added to both samples for viability estimation. Dual-color flow cytometry was performed to quantify the dextran delivery efficiency and cell viability. A delivery efficiency of $\sim 98\%$ along with a post-electroporation cell viability of 89% has been measured reproducible across

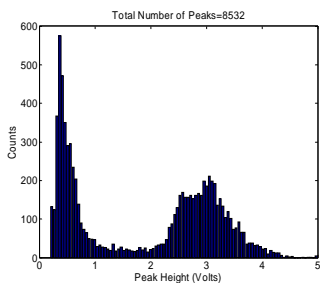


Figure 3. Distribution of voltage peaks obtained with a mixture of polystyrene particles with nominal diameters of 10 μ m and 20 μ m. The mean peak heights roughly scale according to particle volume ($\propto r^3$) as expected theoretically.

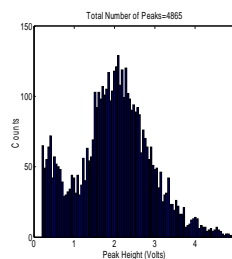


Figure 4. Distribution of voltage peaks obtained with a suspension of NT8e cells (1 \times 10⁵ cells/ml). Compared with the standard particles, using particle volume scaling, the mean cell diameter can be estimated from to be \sim 17 μ m.

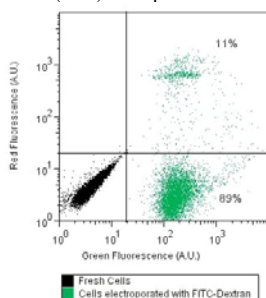


Figure 5. Dual-color flow cytometry results for NT8e cells electroporated with 0.5 mg/ml FITC-Dextran (MW=150kD). The percentage of green fluorescent cells i.e. the delivery efficiency is \sim 98% and the percentage of cells negative in red fluorescence i.e. the post-electroporation cell viability is \sim 89%.

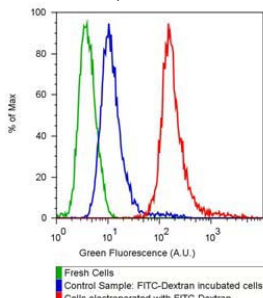


Figure 6. Green-fluorescence intensity distribution for FITC-dextran electroporated cells, control sample cells kept with FITC-dextran and fresh cells. An order of magnitude change in mean fluorescence is seen in the electroporated cells above the non-specific binding in the control cells.

batches of cells. Representative results are shown in Figures 5 & 6.

5. Conclusion

We have developed a novel non-viral delivery method which achieves very high efficiency and post-electroporation cell viability even for a large number of cells. This would be especially useful for applications in which the cells are difficult to culture such as in cancer gene therapy. Experiments are on to demonstrate impedance-selective electroporation which would then enable *ex-vivo* targeted delivery of genes and drugs in mixed suspensions obviating the need for laborious separations and post-transfection selection.

References

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