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Gene delivery by microfluidic flow-through electroporation based on constant DC and AC field*

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Abstract— **Electroporation is one of the most widely used physical methods to deliver exogenous nucleic acids into cells with high efficiency and low toxicity. Conventional electroporation systems typically require expensive pulse generators to provide short electrical pulses at high voltage. In this work, we demonstrate a flow-through electroporation method for continuous transfection of cells based on disposable chips, a syringe pump, and a low-cost power supply that provides a constant voltage. We successfully transfect cells using either DC or AC voltage with high flow rates (ranging from 40 µl/min to 20 ml/min) and high efficiency (up to 75%). We also enable the entire cell membrane to be uniformly permeabilized and dramatically improve gene delivery by inducing complex migrations of cells during the flow.**

I. INTRODUCTION

Gene delivery has been demonstrated as a powerful technique for elucidating gene functions and regulation as well as curing diseases via cell-based therapies. In basic biology studies, the functions of cells could be either enhanced by introducing exogenous plasmid DNA or inhibited by knock-down of specific gene expression using siRNA. Numerous strategies have been developed for gene delivery into cells using viral, chemical, or physical techniques. However, gene transfer using viral vectors or chemicals has safety concerns, and the procedures are complex. These methods also suffer from low efficacy and poor reproducibility.

Electroporation is the most widely-used physical method for delivering genes into cells [1-3]. Electroporation creates nanoscale pores in the cell plasma membrane that permits gene entry by applying an external electric field. Electroporation occurs when the transmembrane potential ($\Delta\psi_E$) exceeds a critical threshold.

$$\Delta\psi_E = 1.5\ g(\lambda) aE\cos\theta \quad (1)$$

where $g(\lambda)$ is a function of the membrane and buffer conductivities, a is the diameter of the cell, E is the field intensity and θ is the angle between the normal to the membrane surface and the field direction. Compared to viral and chemical transfection methods, electroporation offers several important advantages: high transfection efficiency for primary cells, reduced safety concerns, simple operation, and little cell-type dependency [4-6]. Electroporation (or electrofusion) has been investigated at both macro [7-9] or micro scales [10-21]. All available electroporation-based transfection methods require the use of specialized pulse generators to produce short electrical pulses of defined duration and intensity at high voltage [7, 8, 11, 14]. These pulse generators significantly increase the equipment cost and the logistic burden. Moreover, most commercial electroporators work in batch mode using a cuvette with embedded electrodes inside and each batch is restricted to a small volume of sample (~1 mL or ~10^6 cells).

Here we present a family of methods for gene delivery via flow-through electroporation under constant DC and AC voltage. In our method, we use a power supply that applies a constant DC or AC voltage instead of an expensive pulse generator across the fluidic channels. Our technology provides a compelling solution to transfection of cells either in small or large scale for both genetic studies and cell therapy procedures.

II. FLOW-THROUGH ELECTROPORATION BASED ON CONSTANT VOLTAGE

A. Chip Design

The chip is fabricated out of polydimethylsiloxane (PDMS) using standard soft lithography technique. The fluidic channel is composed of a number of alternating wide and narrow sections in order to create variation in the cross-sectional area. The narrow section could be designed as single or multiple. While a constant voltage is established across a channel that is filled with a conductive buffer, based on Ohm's law the field intensities in two sections (E_i and E_j) follow the simple relationship [22]: $E_i/E_j = A_j/A_i$, where A_i and A_j are the cross-sectional areas of these sections, respectively. Due to the planar nature of soft lithography fabrication, the depth of the entire channel is uniform in all the sections and in that case we have: $E_i/E_j = W_j/W_i$, where W_i and W_j are the widths of the sections. The local electrical field strength in each section is inversely proportional to the width of the section under a constant voltage [22]. With appropriate combination of the overall voltage and the channel geometry, only the field intensity in the narrow section(s) is higher than the threshold for electroporation, and low intensity in the wide sections does not affect membrane integrity. Cells experience pulse-like electrical

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field variation(s) while flowing through the channel. The number of the narrow sections determines the number of times that cells are exposed to the electroporation field. By having multiple electroporation sections in series, we are able to deliver a number of pulses to cells with designed pattern and sequence determined by the arrangement of various sections.

We typically have the same width among multiple narrow sections (or multiple wide sections) for the sake of simplicity. In this method, we refer to the width and field intensity of the wide sections as W_1 and E_1 and those of the narrow section(s) as W_2 and E_2, respectively. We also refer to the *combined* length and duration in the wide sections as L_1 and T_1 and those in the narrow section(s) as L_2 and T_2, respectively. The field intensity in these sections (i.e. the electroporation field intensity) is determined by the constant voltage applied across the channel (V) and the geometry of the channel. The residence time of cells in these sections (determined by the cell velocity and the lengths of these sections) determines the electroporation duration. When determining the operational parameters for a device with known dimensions and desired electroporation intensity (E_2) and duration (T_2), we can use the following formulas to calculate the applied voltage (V) and the flow rate of a cell sample (Q):

$$V = E_2(L_1W_2/W_1+L_2) \quad (2)$$

$$Q = W_2L_2H/T_2 \quad (3)$$

where H is the channel depth.

Because the physics does not depend on the absolute size of the device, the channel can be easily scaled up and down by changing the cross-sectional areas of different sections of a channel. We could handle the throughput in the range of micrometers to millimeters to accommodate a processing rate from μl/min to ml/min.

B. System Setup

A typical flow-through electroporation system setup is illustrated in Fig. 1. Cell suspension mixed with plasmid DNA in electroporation buffer (8 mM Na_2HPO_4, 2 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$ and 250 mM sucrose, pH 7.4) is introduced into the PDMS fluidic channel through plastic tubing affixed to the inlet end of the chip, driven by a syringe pump. A power supply is connected to two platinum wire electrodes to provide a constant voltage across the channel. The platinum wires are inserted into the inlet and outlet reservoirs through the PDMS layer. The system is placed in a laminar flow hood during operation. As a proof of principle, we tested the method by delivering pEGFP-C1 plasmid DNA into Chinese hamster ovary (CHO-K1) cells [23, 24]. Transfection efficiency and cell viability were evaluated at 48 h post electroporation.

In a transfection procedure based on flow-through electroporation, the affordable and common instruments including power supply and the syringe pump form the capital equipment. The disposable fluidic chips are the consumable. The cost of the capital equipment in this setup can be easily lower than US$1000 and the unit manufacturing cost of the disposable chip is lower than US$1. Our method is the only electroporation technique based on the application of constant voltage, particularly suitable for continuous production of transfected cells. Such simple requirement eliminates the need for a pulse generator

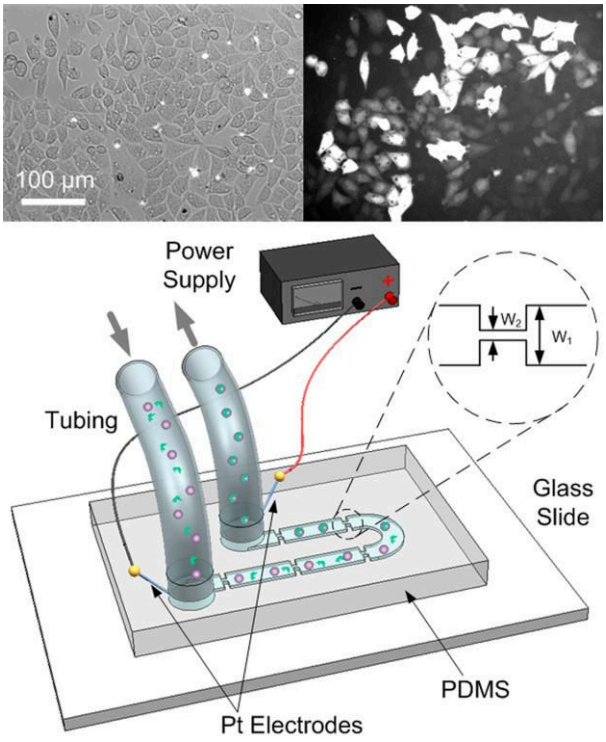

Figure 1. The flow-through electroporation chip and its setup. A chip with 5 narrow sections is shown. The inset images were taken at 48 h after electroporation in a device with 5 narrow sections. Reproduced from ref. 23.

that is used virtually in all other electroporatin systems. We are able to implement sophisticated pulse sequences by variations in the design of the channel. In addition, the utilization of a constant voltage also ensures stable operation and uniform cell treatment over an extensive period of processing.

C. DC-based Gene Delivery

We initially employed a DC power supply in the flow-through electroporation system for transfection of cells. One important advantage of our technique is that the sequence of the electric field variations (equivalent to the pulse pattern in electropulsation) can be conveniently adjusted by altering the geometry of the channel. Therefore, we systematically investigated the effects of the geometry of the channel (i.e. the amount of the narrow sections and the dimension of the wide sections) and the electric parameters (i.e. field intensity, duration and pulse sequence) on transfection by varying the residence time and field intensity in the channel.

The combination of high-intensity and low-intensity pulses has been found to produce the optimal transfection efficiency [25-27]. Multiple pulses created by having multiple narrow sections typically produce superior results to those by devices with one single narrow section [23, 28].

The arrangement of the electroporation sections has significant influence on the transfection efficiency. The narrow electroporation sections are segmented by the wide sections. It was suggested that after the high intensity pulses create pores in the membrane and have DNA molecules bound to the cell surface, the low intensity pulses in the wide sections exerts electrophoretic drag that is important for delivering DNA deeply into the cell interior and facilitates DNA entry into the nucleus. For example, we showed that while the optimal total residence time in the narrow sections T_2 was in the range of 1-10 ms, the combined T_1 in the wide sections needed to be on the order of a few seconds to produce high-efficiency transfection[23]. We also found the transfection efficiency increased substantially with higher field strength in the narrow sections and longer residence time in the wide sections. A ratio of 7-15 for W_1/W_2 ($=E_2/E_1$) guaranteed exclusive electroporation in the narrow sections and no significant compromise of the membrane integrity in the wide sections.

D. AC-based Gene Delivery

We also applied AC field with defined duration and intensity to the flow-through electroporation technique. Low-frequency AC (<10 kHz) produces higher transmembrane potential than high-frequency AC (10k-1M Hz). Furthermore, low-frequency AC (50-60 Hz) is widely used in domestic power systems and may provide drastically simple solution to power need by electroporation. AC pulses for electroporation have conventionally been generated by triggering an AC generator using discrete DC pulses of several milliseconds or shorter. In our work, we generated electropermeabilization and DNA delivery using low-frequency sine-wave or square-wave ac field of 10-10k Hz by modulating a constant AC field across a microfluidic channel. Similar to flow-through electroporation based on DC voltage [22, 23, 29], the AC field intensity in the narrow section was sufficiently high to generate electroporation while the low field in the wide sections did not disrupt the cell membrane.

The results reveal that electropermeabilization becomes weaker with increased frequency in this range. In contrast, transfection efficiency with plasmid DNA reaches its maximum at medium frequencies (100-1000 Hz) in the range. We postulate that the relationship between the transfection efficiency and the AC frequency is determined by combined effects from electrophoretic movement of DNA in the AC field, dependence of the DNA/membrane interaction on the AC frequency, and variation of transfection under different electropermeabilization intensities. AC electroporation in this frequency range yields high efficiency for transfection (up to ~71%), and this result is very comparable to that of DC electroporation. This suggests its potential for gene delivery.

E. Scale-up of Flow-through Electroporation

In clinical cell therapies involving genetically modified cells, ~10^9 cells are typically needed for each trial. A successful scale-up of the flow-through electroporation device will be critical for validating the technique for these clinical procedures.

Since the physics of our flow-through electroporation technique does not depend on the actual size of the device, the device can be scaled up to any dimensions as desired in principle. We have tested channels with feature sizes ranging from tens of micrometers to millimeters with the processing rates between 40 μl/min and 20 ml/min [23]. We typically scale up the devices by increasing the cross-sectional area by the same factor for all the sections in the channel. We need to vary the flow rate of the cell sample accordingly in order to maintain the same field durations. We noticed that the transfection efficiency and cell viability exhibited some dependence on the device size [23]. Such dependence could be substantial when there was only one narrow section in the channel. When channels with one narrow section were used, longer residence times in narrow sections T_2 was required in a larger device in order to yield transfection efficiency comparable to that by a smaller device [23]. This is presumably due to weakened electroporation in a larger device, although the exact physics behind this is not clear. In contrast, channels with multiple narrow sections did not show substantial loss in the transfection efficiency while scaled up due to the improved electroporation efficiency associated with applying multiple pulses [23]. A high number of narrow sections also seem to improve both the transfection efficiency and the cell viability. The optimal electroporation parameters (the field intensity, the residence time, and the combination of narrow/wide sections) appear to have dependence on the device size. When the device size is drastically changed, the optimization of these parameters may need to be repeated. Nevertheless, after optimization of the operational parameters, similar performance can be obtained using devices of different sizes.

F. Vortex-assisted Gene Delivery

One of the major limitations associated with common electroporation is limited delivery area through the cell surface. This is determined by both the physics and the practice of electroporation. Based on (1), the transmembrane potential $\Delta\psi_E$ is highest at the poles of a cell (i.e., $\theta \rightarrow 0$) where the surface normal is aligned with the field direction. When a cell remains static during the application of the electric field (this is typically the case for conventional electroporation given the pulse duration of milliseconds or shorter), permeabilization of the membrane and gene delivery occur mostly at the poles. The rest of the cell membrane is usually not permeabilized under the optimized electroporation conditions (that avoid excessive cell death) [30, 31]. The fact that gene entry into cells is via a small fraction of the cell surface area creates a profound hurdle for improving transfection efficiency.

The flow-through format of our electroporation protocol allows hydrodynamic manipulation of cell motions that strongly influence the cell membrane area being permeabilized. In our recent work, we showed that by inducing transverse Dean flow in the channel with a spiral electroporation section, flow-through electroporation generated permeabilization over the entire cell surface

without introducing significant decrease in the cell viability [24]. Under these flow conditions, cells are involved in both the flow along the channel path and the vortices in the secondary transverse direction (*i.e.* in the plane that is perpendicular to the main flow direction). Such complex migrations expose different surface areas of a cell to the electric field and overcome the major difficulty with conventional electroporation due to limited permeabilized membrane area.

III. CONCLUSION

We present delivery of genes into mammalian cells (e.g. CHO-K1 cells) using the flow-through electroporation technique. By establishing a constant DC or AC voltage across the device, cells experienced pulse-like electrical field variations while flowing in the fluidic channel with alternating sections of large and small cross-sectional areas. The high throughput (up to ~20 mL/min) and high transfection efficiency (up to ~75% demonstrated) could be achieved. These chips could be scaled up to increase the processing volume of the cell sample without compromising transfection efficiency and cell viability. In addition, by harnessing hydrodynamic effects in curved flow paths, cells could be subjected to a combination of transverse vortex motion and rotation, which greatly improves the surface area of cell permeabilication. Compared to the conventional cuvette-based electroporators working in batch mode, our method offers continuous production of transfected cells. By applying low-cost and common lab equipment including a syringe pump, power supplies, and disposable PDMS chips, the system provides a simple and economical method for transfection of cells in either small or large volume.

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REFERENCES

[1] E. Neumann, *et al.*, "Gene transfer into mouse lyoma cells by electroporation in high electric fields," *EMBO J.,* vol. 1, pp. 841-5, 1982.

[2] M. P. Rols, *et al.*, "In vivo electrically mediated protein and gene transfer in murine melanoma," *Nat. Biotechnol.,* vol. 16, pp. 168-71, Feb 1998.

[3] H. Aihara and J. Miyazaki, "Gene transfer into muscle by electroporation in vivo," *Nat. Biotechnol.,* vol. 16, pp. 867-70, Sep 1998.

[4] A. Hamm, *et al.*, "Efficient transfection method for primary cells," *Tissue Eng.,* vol. 8, pp. 235-45, Apr 2002.

[5] P. Lenz, *et al.*, "Nucleoporation of dendritic cells: efficient gene transfer by electroporation into human monocyte-derived dendritic cells," *FEBS Lett.,* vol. 538, pp. 149-54, Mar 13 2003.

[6] F. Andre and L. M. Mir, "DNA electrotransfer: its principles and an updated review of its therapeutic applications," *Gene Ther.,* vol. 11 Suppl 1, pp. S33-42, Oct 2004.

[7] M. P. Rols, *et al.*, "Highly efficient transfection of mammalian cells by electric field pulses. Application to large volumes of cell culture by using a flow system," *Eur. J. Biochem.,* vol. 206, pp. 115-21, May 15 1992.

[8] T. C. Bakker Schut, *et al.*, "Selective electrofusion of conjugated cells in flow," *Biophys. J.,* vol. 65, pp. 568-72, Aug 1993.

[9] L. H. Li, *et al.*, "Highly efficient, large volume flow electroporation," *Technol. Cancer Res. Treat.,* vol. 1, pp. 341-50, Oct 2002.

[10] S.-W. Lee and Y.-C. Tai, "A micro cell lysis device," *Sens. Actuators A,* vol. 73, pp. 74-79, 1999.

[11] Y.-C. Lin and M.-Y. Huang, "Electroporation microchips for in vitro gene transfection," *J. Micromech. Microeng.,* p. 542, 2001.

[12] Y.-C. Lin, *et al.*, "Electroporation microchips for continuous gene transfection," *Sens. Actuators B,* vol. 79, pp. 137-143, 2001.

[13] Y. Huang and B. Rubinsky, "Microfabricated electroporation chip for single cell membrane permeabilization," *Sens. Actuators A,* vol. 89, pp. 242-249, 2001.

[14] Y. Huang and B. Rubinsky, "Flow-through micro-electroporation chip for high efficiency single-cell genetic manipulation," *Sens. Actuators A,* vol. 104, pp. 205-212, 2003.

[15] Y. C. Lin, *et al.*, "Simulation and experimental demonstration of the electric field assisted electroporation microchip for in vitro gene delivery enhancement," *Lab Chip,* vol. 4, pp. 104-8, Apr 2004.

[16] N. R. Munce, *et al.*, "Microfabricated system for parallel single-cell capillary electrophoresis," *Anal. Chem.,* vol. 76, pp. 4983-9, Sep 1 2004.

[17] H. Lu, *et al.*, "A microfluidic electroporation device for cell lysis," *Lab Chip,* vol. 5, pp. 23-9, Jan 2005.

[18] M. Khine, *et al.*, "A single cell electroporation chip," *Lab Chip,* vol. 5, pp. 38-43, Jan 2005.

[19] Z. Fei, *et al.*, "Gene transfection of mammalian cells using membrane sandwich electroporation," *Anal. Chem.,* vol. 79, pp. 5719-22, Aug 1 2007.

[20] A. Valero, *et al.*, "Gene transfer and protein dynamics in stem cells using single cell electroporation in a microfluidic device," *Lab Chip,* vol. 8, pp. 62-7, Jan 2008.

[21] A. M. Skelley, *et al.*, "Microfluidic control of cell pairing and fusion," *Nat. Methods,* vol. 6, pp. 147-52, Feb 2009.

[22] H. Y. Wang and C. Lu, "Electroporation of mammalian cells in a microfluidic channel with geometric variation," *Anal. Chem.,* vol. 78, pp. 5158-64, Jul 15 2006.

[23] T. Geng, *et al.*, "Flow-through electroporation based on constant voltage for large-volume transfection of cells," *J. Control. Release,* vol. 144, pp. 91-100, May 21 2010.

[24] J. Wang, *et al.*, "Vortex-assisted DNA delivery," *Lab Chip,* vol. 10, pp. 2057-61, Aug 21 2010.

[25] M. F. Bureau, *et al.*, "Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer," *Biochim. Biophys. Acta,* vol. 1474, pp. 353-9, May 1 2000.

[26] S. Satkauskas, *et al.*, "Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis," *Mol. Ther.,* vol. 5, pp. 133-40, Feb 2002.

[27] S. Satkauskas, *et al.*, "Electrophoretic component of electric pulses determines the efficacy of in vivo DNA electrotransfer," *Hum. Gene Ther.,* vol. 16, pp. 1194-201, Oct 2005.

[28] H. Y. Wang and C. Lu, "Microfluidic electroporation for delivery of small molecules and genes into cells using a common DC power supply," *Biotechnol. Bioeng.,* vol. 100, pp. 579-86, Jun 15 2008.

[29] T. Geng, *et al.*, "Transfection of cells using flow-through electroporation based on constant voltage," *Nature Protoc.,* vol. 6, pp. 1192–1208, 2011.

[30] E. Tekle, *et al.*, "Selective and asymmetric molecular transport across electroporated cell membranes," *Proc. Natl. Acad. Sci U.S.A.,* vol. 91, pp. 11512-6, Nov 22 1994.

[31] M. Golzio, *et al.*, "Direct visualization at the single-cell level of electrically mediated gene delivery," *Proc. Natl. Acad. Sci U.S.A.,* vol. 99, pp. 1292-1297, Feb 5 2002.