Biomed Microdevices (2008) 10:113–116
DOI 10.1007/s10544-007-9115-x

Electrophoresis-assisted single-cell electroporation for efficient intracellular delivery

Cristian Ionescu-Zanetti · Andrew Blatz · Michelle Khine

Published online: 8 September 2007

Abstract Single-cell electroporation, in which a focused electric field is applied to permeabilize an individual target cell using relatively low applied voltages, has demonstrated improved cell viability and transfection rates over conventional bulk electroporation set-ups. Here, we introduce a new strategy, in conjunction with single-cell electroporation, to enhance exogenous transport efficiency: electrophoresis delivery of compounds subsequent to electroporation. Electrophoresis is used to assist loading of otherwise impermeable exogenous anionic fluorescent molecules Calcein (Invitrogen, MW=622) and Oregon Green Dextran (OGD, Invitrogen, MW=70,000). For the larger dextran molecules, we demonstrate a protocol of first preconcentrating at the cell-microfluidic channel interface. Then, the electric field is used to drive these molecules into the cell post-electroporation using 50–200 mV. We demonstrate delivery rate enhancements of more than an order of magnitude using electrophoresis compared to diffusion alone subsequent to electroporation.

Keywords Single-cell electroporation · Electrophoresis · Microfluidics

C. Ionescu-Zanetti · A. Blatz · M. Khine
Fluxion Biosciences,
San Francisco, CA 94158, USA

M. Khine (✉)
School of Engineering, University of California, Merced,
Merced, CA 95343, USA
e-mail: mkhine@ucmerced.edu

1 Introduction

The rapid yet well-controlled introduction into, or extraction from, individual cells of otherwise impermeable compounds (e.g., drug candidates, RNA, DNA, proteins) is a pervasive challenge in basic cell biology research. Most methods rely on addressing a large population of cells in bulk. Traditional bulk electroporation, in which thousands of volts are applied across millions of cells, offers little control over the permeabilization of individual cells. As a consequence, reversible electroporation, in which the cells reseal and remain viable, is difficult to achieve (Kanduser et al. 2006). Moreover, controlled quantitative dosing of exogenous compounds into the cells is not possible. From the extraction point of view, the ability to analyze the contents of individual cells, instead of pooling from a population of disparate cells, is critical for investigating the distribution of various critical parameters between cells (DiCarlo et al. 2006; Rao et al. 2002). Even cells that are identical genetically exhibit marked variations in gene expression and cellular response. Heterogeneous single cell behavior, apparent even when the population's behavior is predictable and reproducible, implies that this cannot be described purely deterministically (Ko et al. 1990). The ability to analyze the contents of single cells is therefore an important challenge in capillary electrophoresis (Stuart et al. 2003). Electroporation obviates the need for chemical lysis and if the cells can be isolated, as in this configuration, prevents cross contamination between individual cell contents.

Previously, we have developed a bench-top single-cell electroporation array system comprised of a control interface, with mating disposable 96-well-based microfluidic chips, that enables cells to each be controlled, monitored and individually manipulated (Khine et al. 2005,

2007). Our minimally invasive approach, using a feedback control loop which releases the electric field immediately after electroporation, enables improved membrane resealing kinetics for greater percentage cell viability. However, a consequence of the rapid resealing is the limited amount of material that can be delivered into the cell by diffusion. We therefore introduce here a new strategy to improve exogenous transport efficiency: electrophoresis delivery of compounds subsequent to electroporation. Electrophoresis can be used to assist loading by first pre-concentrating molecules of interest at the cell-channel interface. Then, the electric field is used to drive these molecules into the cell post-electroporation. In this proof of principle, we demonstrate the feasibility of electroporating an array (n=15) of Hela cells in suspension and inserting otherwise impermeable Calcein (Invitrogen, MW=622) and Oregon Green Dextran 514 (Invitrogen, MW=70,000) by electrophoresis.

Moreover, in our configuration, extracting the content of one cell per well would thus be feasible by operating in reverse mode. Because the cells in our configuration are each attached to their own capillary, cross contamination between channels is avoided (Fig. 1). This technology, therefore, can be leveraged to extract the contents of single cells and can be eventually combined with fluorescence labelling for high-sensitivity detection (Gilman et al. 1995; Munce et al. 2004).

2 Experimental

The bench-top 96-well format microfluidic array for single-cell electroporation is detailed elsewhere [8]. Briefly, a bottomless 96-well plate is divided into four quadrants and each bonded to a disposable microfluidic polydimethylsiloxane (PDMS, Dow Corning) chip with lateral channels which terminate in 3×3 μm capillaries for the cell trapping and electric field focusing (Fig. 1). Each capillary channel is pneumatically and electrically independently addressable and connects to its own well on a standard size 96-well plate (bottomless 96-well plates, Nalge Nunc International). Hela (human cervical cancer) cells in suspension are loaded into the device, trapped in its capillary channel by slight transient negative pressure, and the voltage is applied through a modified patch clamp amplifier (the Warner 505B Patch Clamp Amplifier).

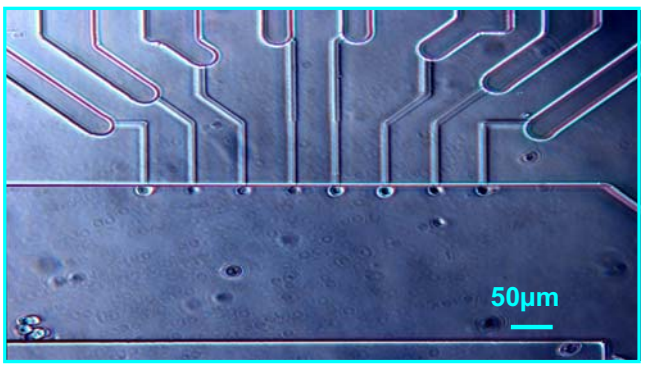

Fig. 1 Array (n=8) of Hela cells trapped. Bright field close up of upper half of viewing window (with n=15 cells). Each cell is interfaced with a capillary channel that is electrically and pneumatically individually addressable and connected to the bottom of a single well of a 96-well plate

The capillary channels are preloaded with the exogenous anionic fluorescent molecules. For the larger molecules, we implement a protocol to enhance delivery rate (Fig. 2). An electric field (0–300 mV) that is below the electroporation threshold (0.5–2 V) can be applied to the cell trapping

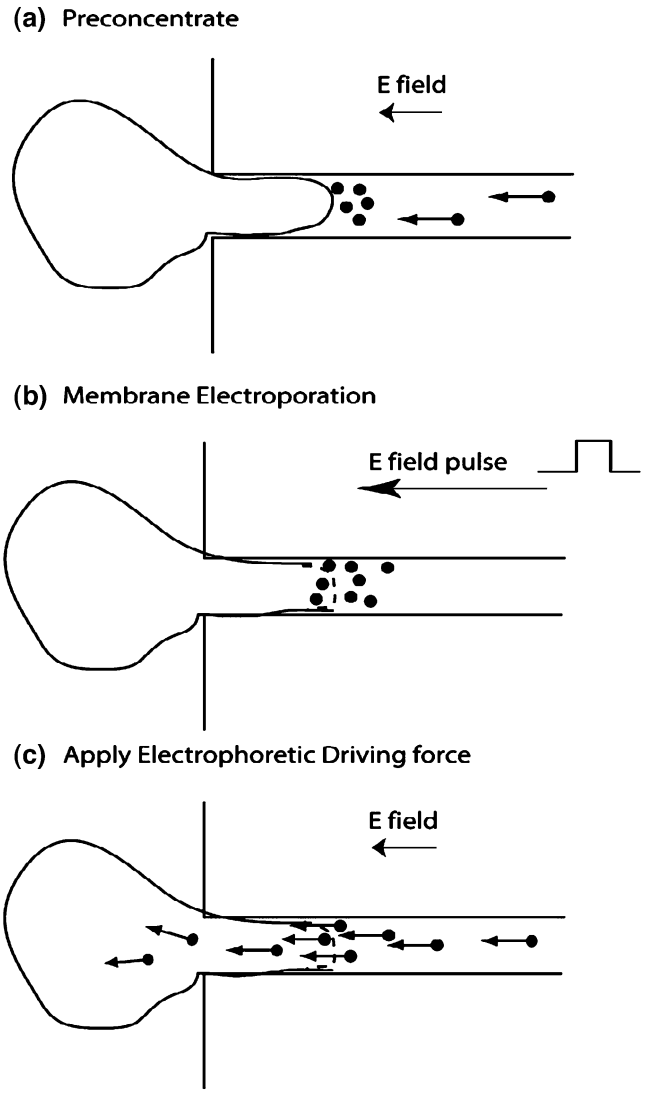

Fig. 2 Schematic of electrophoresis-driven cell loading protocol. An optional preconditioning step before the cell membrane is porated is preconcentration (**a**). A small field is used to increase molecule concentration next to the membrane patch to be porated. In (**b**) a short high intensity pulse is used to electroporate the membrane. During resealing a constant low field is used again to drive molecules into the cytoplasm (**c**)

microchannel after the positioning of a cell pneumatically at the opening. Cells must be positioned in such as way as to ensure the cell membrane remains intact, this can be done using a slight negative pressure. After accumulation of the species of interest for a set period of time (verified by fluorescence intensity), the cell is electroporated by applying a larger amplitude square wave (5–30 ms) [Fig. 2(b)]. Subsequently loading into the cell can be driven via electrophoresis during the resealing period post-electroporation [Fig. 2(c)].

3 Results and discussion

Holding the cell at −200 mV immediately after electroporation enables Calcein loading into the cell within 3 seconds (Fig. 3), which by diffusion alone took upwards of 16 s (data not shown). Using the protocol, we demonstrate we can even load cells that reseal quickly after the electroporation event. The duration and intensity of the electrophoretic field can be furthermore used to control and concentrate the amount of material inserted, leading to dose control. Moreover, this enables the delivery of even larger molecules (MW=70,000) into the cell in a time scale that could enable the cell to remain viable. Using diffusion loading alone, it took upwards of 30 min to load the cell with OGD. This obviously is not an acceptable time scale for intracellular delivery. Conversely, when an electrophoretic field is applied (−100 mV intracellular), we demonstrate routine loading of OGD within 40 s (Fig. 4). It should be noted that we only worked with charged exogenous compounds, but it may be possible to use the amplifier to charge neutral particles prior to loading.

4 Conclusion

We have demonstrated electrophoresis-driven loading in two different regimes: pre-concentration and post-electroporation loading. We also demonstrate loading by post-electroporation electrophoresis without the aid of pre-concentrating at the cell interface first. Using this approach, a single cell was loaded with fluorescent calcein molecules over a period of only 3 s as shown in Fig. 3. Pre-concentration of OGD at the electroporation site (next to the cell membrane) was performed by applying a constant field to the trapping capillary (50–100 mV). After the electroporation event, a constant field

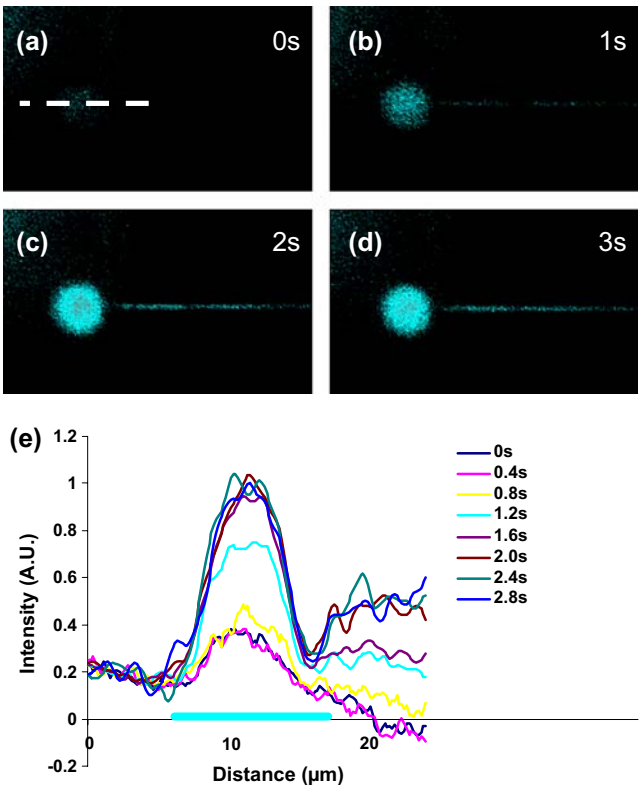

Fig. 3 Fast calcein loading. (**a**–**d**) Within a 3 sec time span, membrane impermeable Calcein is loaded into the cell using an electrophoretic driving force. (**e**) The cell intensity measured across a cell section [*dotted line* in (**a**)]

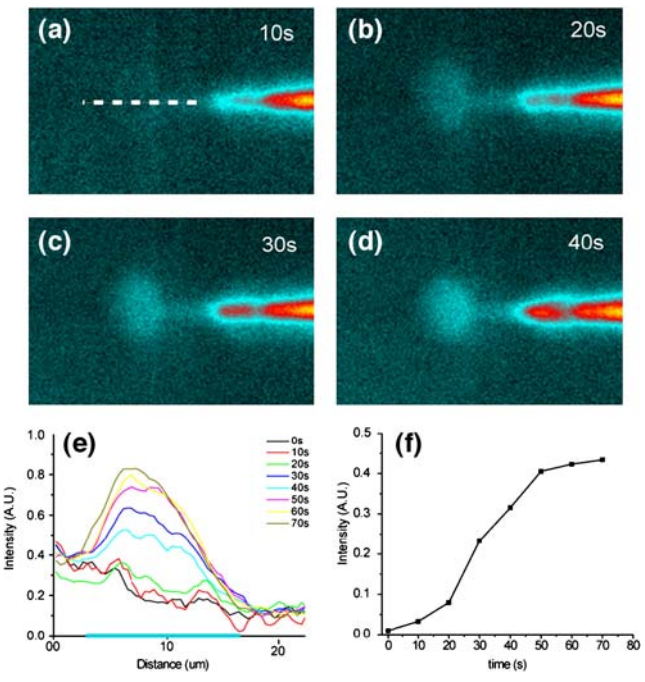

Fig. 4 Fast OGD loading. (**a**–**d**) Within a 1 min time span, labeled dextran have been loaded into the cell using a electrophoretic driving force. (**e**) The cell intensity measured across a cell section (*dotted line* in **a**). (**f**) Integrated cell intensity as a function of time. EP event was measured dat the 10 s time point

was applied in order to enhance loading. Fluorescent imaging data of the time-resolved loading of a single cell after electroporation using this approach is shown in Fig. 4. The loading time was shortened to below a minute as compared to >30 min using diffusion alone. Moreover, electrophoresis resulted in the ability to load more material into the cell than by diffusion alone.

In conclusion, we have successfully used smaller fields post-electroporation to load exogenous fluorescent molecules of various sizes into single cells. Variation in field intensity and duration both before and after the electroporation protocol can be used to control the amount of material inserted (dosing). The difference in load rate between no potential and a −200 mV applied voltage is approximately 40× for large molecules. The next step is to demonstrate the extraction of intracellular single-cell contents into the attached well-plate using the same setup.

Acknowledgments Funding Source: National Institute of Health NIGMS 1 R43 GM075509-01.

References

D. DiCarlo, N. Aghdam, L.P. Lee, Anal. Chem. **78**(14), 4925–4930 (2006)
S.D. Gilman, A.G. Ewing, Anal. Chem. **67**, 58–64 (1995)
M. Kanduser, M. Sentjurc, D. Milkavcic, Eur. Biophys. J. **35**, 196–204 (2006)
M. Khine, A. Lau, C. Ionescu-Zanetti, J. Seo, L.P. Lee, Lab Chip, **5** (1), 38–43 (2005)
M. Khine, C. Ionescu-Zanetti, A. Blatz, L.P. Wang, L.P. Lee, Lab Chip, **7**(4), 457–462 (2007)
M.S. Ko, H. Nakauchi, N. Takahashi, EMBO J. **9**, 2835–2842 (1990)
N.R. Munce, J. Li, P.R. Herman, L. Lilge, Anal. Chem. **76**, 4983–4989 (2004)
C.V. Rao, D.M. Wolf, A.P. Arkin, Nature **420**, 231–237 (2002)
J.N. Stuart, J.V. Sweedler, Anal. Bioanal. Chem. **375**(1), 28–29 (2003)