Lab on a Chip

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**PAPER**

# Droplet electroporation in microfluidics for efficient cell transformation with or without cell wall removal†

**Baiyan Qu,[a] Young-Jae Eu,[a] Won-Joong Jeong[b] and Dong-Pyo Kim*[c]**

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An efficient cell transformation method is presented that utilizes droplet electroporation on a microfluidic chip. Two types of green microalgae, a wall-less mutant and a wild type of *Chlamydomonas reinhardtii*, are used as model cells. The PDMS-glass electroporation chip is simply composed of a flow-focusing microstructure for generating cell-encapsulating droplets and a serpentine channel for better mixing of the content in the droplet, and five pairs of parallel microelectrodes on the glass slide, without involving any expensive electrical equipment. The transformation efficiency *via* the microfluidic electroporation is shown to be more than three orders of magnitude higher for the wall-less mutant, and more than two orders of magnitude higher for the wild type, which has its cell wall intact, than bulk phase electroporation under identical conditions. Furthermore, the microfluidic transformation is remarkably efficient even at a low DNA/cell ratio, facilitating ways of controlling the transgenic copy number, which is important for the stability of the transgene expression.

## Introduction

Microalgae, ranging in size from 1 μm to over 2 mm, have received much of the spotlight lately because of their potential for producing algae oil as an alternative to fossil fuels and their uses in a number of industrial applications, including bioremediation, high-valued chemicals and pharmaceuticals. Furthermore, they can also be used as a means of controlling pollution and harnessing unwanted $CO_2$.[1] The ability to genetically engineer microalgae, and eventual manipulation of their metabolic pathway, would greatly enhance their usefulness as a scientifically and industrially important species for production of the desired materials. Therefore, development of nuclear transformation techniques is focused on generating transgenic microalgae strains of commercial and scientific value.[2] *Chlamydomonas reinhardtii*, a unicellular green microalga, is the most widely used species as a model organism for fundamental research in cell and molecular biology, and as a source of bioenergy, such as hydrogen and biodiesel.

Nuclear transformation protocols of *C. reinhardtii* are also broadly established for generating transgenic strains, such as particle bombardment,[3,4] electroporation[5] and agitation with glass beads[6] or silicon carbide whiskers.[7] Among them, electroporation and vortexing with glass beads are the most popular methods. In particular, electroporation achieved a maximum transformation of $\sim 2 \times 10^5$ transformants per μg of DNA, which is two orders of magnitude higher than what is obtained with the glass bead.[5,8]

In bulk electroporation, millions of cells in suspension were subjected to a high applied voltage (typically kV) to reach the requisite transmembrane potential. But the Joule heating, as well as uncontrolled and non-homogenous drops in the field across different cells, resulted in a low cell viability.[9] And delivering the same dose of exogenous compounds into all the cells is still a challenging task.[9] Furthermore, transgene silencing due to multi-copy insertion of a transgene into the genome was often reported in most of the eukaryotic organisms including *C. reinhardtii*.[10–12]

Microfluidics is ideally suited for single cell electroporation, because it can be used to overcome the inherent drawbacks of bulk electroporation. First of all, only a relatively low potential is needed to generate a high electric field strength with microelectrodes. Cell handling and manipulation is easier due to the dimensions of the channels and electrodes, which are comparable to the cell size. Furthermore, heat dissipation is fast, owing to the large surface area-to-volume ratio.[8] These advantages, offered by microfluidics, open various avenues of optimizing the transformation conditions, resulting in improved transformation efficiency. In fact, fabricated microstructures such as micro-holes (2 to 10 μm diameter),[13,14] a filter-like microstructure (4 μm width trapping sites),[15] trapping channels (3.1 μm height),[16] and a 96-well plate[17] have been used to trap the single cell for electroporation. However, all of these studies involved one-at-a-time electroporation and required complicated

[a]*Department of Fine Chemicals Engineering and Chemistry, Chungnam National University, Daejeon, 305-764, Korea.*
[b]*Plant Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, 305-806, Korea*
[c]*Center of Applied Microfluidic Chemistry, Department of Chemical Engineering, Pohang University of Science & Technology, Pohang, 790-784, Korea. E-mail: dpkim@postech.ac.kr*
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microfabrication techniques with high precision, as well as an expensive pulse power supply for electroporation. Therefore, the productivity is low and they are not suitable for continuous experiments.

In recent years, droplet microfluidics has showed promising breakthroughs in biotechnology applications. For example, animal cell growth in an encapsulated droplet has been shown to yield high viability in a picoliter-sized microfluidic drop.[18–21]

Here, we have reported a continuous electroporation method using the droplet microfluidic technique, and demonstrated that the method yields a remarkably high transformation efficiency, even when the cell wall is not removed. Both the wild-type cell with a cell wall and the wall-less strain of microalga, *C. reinhardtii*, were used as model cells. The high transformation efficiency made possible even at a low DNA/cell ratio would eventually lead to ways to effectively control the transgenic copy number.

The algal cell and a controlled number of DNA are encapsulated into an aqueous droplet using a simple channel on a glass slide patterned with electrodes; then the cell-containing droplet flows in a continuous oil phase across 5 pairs of microelectrodes biased with a constant voltage. It is worth noting that there has been no report of continuous direct electroporation of wild-type plant cells, which have a cell wall and a cell membrane, without the cell wall removal step, while most animal cells, having only a cell membrane, can readily be transformed at low electric field strength either by a bulk or a microfluidic process.[15,23] Therefore, droplet microfluidic electroporation can be useful for efficient transformation of algal cells, as well as other microorganisms that are difficult to transform by bulk electroporation under harsh conditions.

## Materials and methods

### Principle of operation

An electric current runs through the conductive buffer of the droplet while it passes across the electrodes, to electroporate the encapsulated cells.[24] When the non-conductive oil is on the electrodes, the electric current is broken until the next droplet passes through them to "reopen" the electric current for the next cycle. The schematic diagram is shown in Fig. 1.

A fluoro continuous oil phase, which is a clear, colorless, thermally and chemically stable, practically non-toxic and fully-fluorinated liquid, is used to avoid the residual oil layer covering the electrodes, which can prevent droplets from making electrical contact.

The electroporation time, during which a droplet is touching a pair of electrodes, can be controlled by the flow rates of the oil and the droplet containing the cell and DNA. When the flow rates of oil and the cell/DNA droplet are 0.5 μL $min^{-1}$ and 0.3 μL $min^{-1}$, respectively, the electroporation time is only 7 ms. Continuous DC power is applied to the electrodes in this experiment to produce an electric field during the time of electroporation, instead of using an expensive pulse power supply. The short distance of 33 μm between electrodes for each pair allowed a very low voltage of 1.3 V to produce a 393 V $cm^{-1}$ electric field for electroporation of the algal cell using the DC power supply.

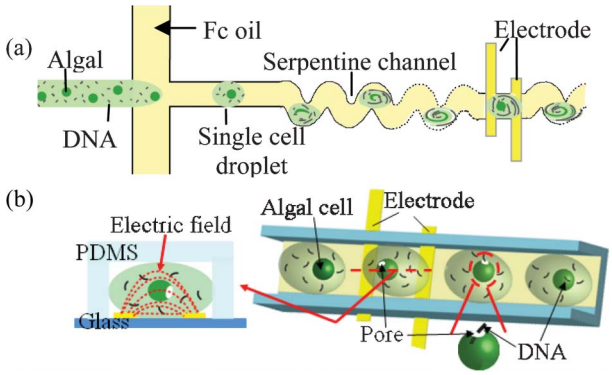



**Fig. 1** Principle diagram of droplet microfluidic electroporation: (a) schematic diagram, (b) electroporetic process of the algal cell. (Not according to the actual scale.)

### Methods

### Device fabrication

**1. PDMS slide.** The PDMS slide was fabricated by the standard soft lithography method. First, the microscale patterns were created using computer-aided design software (AutoCAD) and then printed out on a high-resolution transparency. SU-8 50, a negative photoresist, diluted to 90% with developer pGMEA (1-methoxy 2-propyl acetate), was spun on a 3 inch silicon wafer to generate a layer of 18 μm thickness. After two-step prebaking at 75 °C for 5 min and at 105 °C for 3 min, it was exposed to UV light through the photomask to transfer the patterns, and the SU-8 master on the Si wafer was obtained by a consecutive post-baking and developing step. PDMS prepolymer mixture consisting of the monomer and a curing agent, in the ratio 15 to 1, was cast on the master and heated at 70 °C for about 30 min to consolidate the PDMS, then it was peeled off and punched to create inlet and outlet holes. The PDMS slide is composed of two parts: a flow-focusing part to generate droplets for the encapsulation of cell(s), and a serpentine channel part to enhance

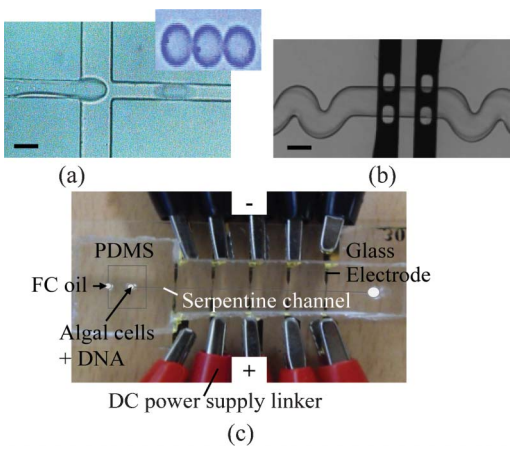



**Fig. 2** Algal cell electroporation on a microfluidic chip: optical image of (a) droplet generation and a single cell encapsulated in a droplet, (b) serpentine channel and electrodes, and (c) whole electroporation chip system. The scale bar is 50 μm.

the chaotic mix in the droplet, to increase DNA access to the cell (shown in Fig. 2(a) and (b)).

**2. Microelectrode glass slide.** There are five pairs of parallel golden microelectrodes along the microchannel on the glass slide. For the fabrication of surface microelectrodes, an Au layer of 200 nm in thickness was deposited onto cleaned glass slides using an E-beam evaporator (KVET-C500200) with 30 nm thickness of Ti for stronger adhesion between the glass and the gold. Positive photoresist AZ 1512 was coated on the Au/Ti glass substrate followed by the conventional photolithographic process with a patterned mask film. The exposed Au/Ti part was then serially wet etched using gold etchant ($KI : I_2 : H_2O$ = 4 g : 1 g : 40 ml) and Ti etchant (10% oxalic acid at 75–85 °C). Finally, the remaining photoresist was removed by acetone to yield 5 pairs of serial Au/Ti microelectrodes with a separation of 5.78 mm on the glass slide. A pair of electrodes consisted of two electrodes that were 50 μm wide and 33 μm apart them (the whole chip system is shown in Fig. 2(c)).

**3. Microfluidic device fabrication.** Both the PDMS microchannel (cross-sectional dimensions 50 μm width × 22 μm height), and the electrode/glass slide were carefully aligned under a microscope (as shown in Fig. 2(b)) and bonded together after the PDMS surface was oxidized with a plasma cleaner. To attain strong bonding and completely cured PDMS, the whole device was finally baked at 80 °C overnight. Subsequently, the channel was flushed with a commercial surface-coating agent (Aquapel, PPG industries), then dried with air to obtain the hydrophobic glass surface to avoid the cell sticking to the glass by repeating the coating–drying step 3 times.

### Experimental procedure

Two strains of *C. reinhardtii*, cc-400 (cw-15 $mt^+$, cell wall-less) and cc-124 (wild-type $mt^-$), were cultured under continuous light in the medium of tris-acetate-phosphate (TAP)[25] to a density of $2 \times 10^6$ cells $ml^{-1}$. Then the cells were collected by centrifugation at room temperature at 3500 rpm for 5 min in a sterile centrifugation tube. The specific number of cells, counted by a hemocytometer, were re-suspended in the electroporation buffer (65.5% polyvinylpyrrolidone silica (Percoll®, Sigma) and 40 mM sucrose in TAP) to render two cell solutions: $1.58 \times 10^7$ cells $mL^{-1}$ and $3.96 \times 10^6$ cells $mL^{-1}$. A specified volume of the DNA fragments of pHyg3[26] with the aph7″ gene conferring hygromycin B resistance were added into the cell solution to control the number ratio of DNA to cells.

For electroporation on a chip, the cell/DNA suspension as a dispersion phase was injected into the microfluidic chip at a flow rate of 0.3 μL $min^{-1}$ with a syringe pump. The FC-70 oil with 5% surfactant (1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol, Sigma) as a continuous phase was injected at a flow rate of 0.5 μL $min^{-1}$ to generate the droplets containing algae cell(s). A DC power supply was used for algae electroporation. The large number of cells ($>10^6$) collected in the outlet were dispersed in 1 mL of 20% starch containing TAP medium, 40 mM sucrose and 0.4% (w/v) PEG 8000 (Sigma) after washing with TAP solution. 2–3 weeks of cultivation yielded colonies in the selective plate (1.5% agar containing TAP and 15 mg $L^{-1}$ hygromycin B (A.G. Scientific, Inc.)). The single colony was further cultured in the selective liquid TAP medium. The DNA of the culture was extracted by the SDS/phenol method for PCR detection of the transgene (the detail of PCR detection is described in the ESI,† with image). Then the transformation efficiency was calculated from the ratio of the number of positive colonies to the total number of cells used in electroporation.

For comparison, the bulk phase electroporation was performed as a control under the same conditions as applied for the microfluidic chip. The cell/DNA solution in a 2 mm cuvette was transformed using a commercial electroporator (Bio-Rad) under conditions of 0.75 kV and 25 μF (the details of the process are described in the ESI†).

## Results and discussion

### Electroporation of cell wall-less algal cell

Droplet microfluidics is a very efficient tool for confining a specific number of cells under independent circumstances. In this work, the wall-less mutant strain CC-400 (cw15 $mt^+$) of *C. reinhardtii* was initially used for demonstrating the electroporetic transformation in the microfluidic chip. Then the cell walled strain CC-124 (wild-type $mt^-$) made of hydroxyproline-rich glycoproteins was finally tested under optimized conditions, as described in the next section.

Cell encapsulating droplets were obtained by controlling the flow rates of the dispersion phase (DNA/cell solution) and the continuous phase (FC-70 oil, 3 M Fluorinert) as shown in Fig. 2(a). The ratio of cell-containing droplets became *ca.* 70% when a concentration of $1.58 \times 10^7$ cells $mL^{-1}$ was used, while the ratio rapidly decreased to *ca.* 20% upon lowering the cell-loading concentration to $3.96 \times 10^6$ cells $mL^{-1}$ (Table 1). Thus, it was appropriate to use a cell-loading concentration of $1.58 \times 10^7$ cells $mL^{-1}$ to achieve reasonable transformation productivity. In addition, the use of a polyvinylpyrrolidone silica additive with high density and low viscosity (10 cP at 20 °C) into the electroporation buffer promoted long term dispersion of the alga cells by preventing them from gravitationally settling down prior to droplet formation. The encapsulation of the cell in the droplet was facilitated by using the buoyant buffer with low osmolarity and no toxicity towards the cells and their constituents.

For actual electroporation on a chip, the algal cell was mixed with a specified volume of the DNA gene conferring hygromycin B resistance in a controlled manner. The DNA/algal cell suspension as a dispersion phase was injected into the microfluidic chip to generate the encapsulating droplets. The electrically conducting drop containing DNA and the cell mixture in the buffer solution passes across a pair of electrodes

**Table 1** Dependence of cell-loading concentration in the buffer on the formation ratio of cell encapsulating droplets, performed with the cell wall-less mutant strain CC-400 (cw15 $mt^+$)

| | Encapsulation ratio (%) | | | | | |
|---|---|---|---|---|---|---|
| | Cell number per droplet | | | | | |
| Cell concentration in the buffer ($mL^{-1}$) | 0 | 1 | 2 | 3 | 4 | 5 |
| $1.58 \times 10^7$ | 31.4 | 38.5 | 22.5 | 6.3 | 1.1 | 0.2 |
| $3.96 \times 10^6$ | 79.1 | 18.8 | 2.1 | 0 | 0 | 0 |

that provide an electric shock for the DNA insertion into the cell, while electrically insulating the continuous oil phase flow across electrodes does not lead to any electric interaction. In addition, the maximum transmembrane potential for on-chip electroporation is about $\pm 295$ mV, which was in the reported range 200–450 mV for eukaryotic cells with the common feature of a bilayer membrane (see ESI†).[27,28] The volume fraction of the cell (spherical shape, 10 μm dia.) to the droplet (63 μm length, channel cross-section 50 μm width × 22 μm height) is about 1/100 as a scaling factor for on-chip electroporation.

It is well documented that the transgenic copy number is known to be related to the stability of the transgene expression.[5] In bulk phase electroporation, multi-copy insertion of the transgene into the genome often tends to cause gene silencing, resulting in low or no expression of the transgene. Therefore, it is desirable to control the copy number for a stable expression of transgene in transformants. In the present work, the ratio of DNA to algal cell was controlled to investigate the effect of the electroporation efficiency. Besides the ratio of $5.7 \times 10^6$ DNA/algal cell corresponding to DNA (40 μg ml$^{-1}$) for $10^8$ cells in 0.25 ml, which is often used for bulk phase electroporation, much lower DNA/algal cell ratios such as 1000, 500 and 100 were comparatively tested by electroporation both in the bulk phase and in droplet microfluidics, as shown in Table 2. The results in the table are generally consistent with those reported in the literature, in that a high DNA/cell ratio yields a high transformation efficiency for both cases of chip and bulk phase electroporation. It should be pointed out, however, that electroporation on a chip produced a much higher transformation efficiency than the commercial electroporator in the bulk phase. At a DNA/cell ratio of $5.7 \times 10^6$, the transformation efficiency of on-chip electroporation was about 100 times higher than that in the bulk phase. In the case of a DNA/cell ratio of 1000, the transformation efficiency for electroporation on a chip was $8.14 \times 10^{-4}$, which was more than 1600 times higher than the value of $5.05 \times 10^{-7}$ in bulk phase electroporation. Furthermore, the transformation efficiency of on-chip electroporation increased about 387 times when the DNA/cell number increased only 5 times from 100 to 500. However, an increase in efficiency of about the same order of magnitude is obtained, even when the DNA/cell number is drastically increased from 1000 to $5.7 \times 10^6$. The comparison reveals that the on-chip electroporation is a more efficient transformation tool at a low range of DNA copy number, presumably due to the high mixing efficiency in the droplet. These results indicate that on-chip electroporation using a picoliter volume of droplet is more sensitive to small amounts of DNA and produces a higher transformation efficiency than bulk phase electroporation. In addition, the *C. reinhardtii* cells in this study were asynchronously grown in continuous light in the TAP medium, which means that at any given time a good number of algal cells will be in the cell cycle that have just recently divided. Thus, the algal cells might be more susceptible to being electroporated, which might contribute to a high transformation efficiency.

The DNA/cell mixture confined in discrete droplets on a picoliter scale that moves along the channel gets homogenized quickly in the distribution, through chaotic advection of the droplets, which greatly enhances the degree of mixing, as has been well-documented.[30,31] This enhanced mixing could increase the probability of a DNA molecule coming into contact with cells passing the electrodes as a high transmembrane potential zone, facilitating the transformation even under a somewhat non-uniform electric field in a droplet in which the region near the electrodes on the channel floor has a higher intensity than that near the channel roof. Furthermore, the high surface area-to-volume ratio of the droplet would result in rapid mass and heat transfer, which was effective in promoting chemical performance, in comparison to the bulk process. In contrast, the cells in bulk phase electroporation are subjected to non-homogeneous distribution under inefficient mixing conditions, and a mutual shielding effect among cells. It is of interest to note in the table that the electrodes on the serpentine channel showed a ~3 times higher transformation efficiency than the electrodes on the straight channel (Table 2 and Table S3, ESI†). It is also consistent with the report that the serpentine channel created further chaotic mixing inside the droplets than the straight channel.[32,33]

It is well known that strong electric pulses to cells cause structural rearrangement of the cell membrane, which leads to the formation of temporary aqueous pathways (“pores”), allowing ionic and molecular (*e.g.* DNA) movement through the pores.[29] While the shocks create pores, they can be harmful for cell viability. To test the effects of the number of shocks on

**Table 2** Effect of DNA/algal cell ratio and electroporation techniques on transformation efficiency, performed with a CC-400 cell-loading concentration of $1.58 \times 10^7$ cells mL$^{-1}$, and a serpentine channel with five pairs of parallel electrodes

| Transformation | DNA/algal cell ratio[a] | Transformation efficiency |
|---|---|---|
| On chip | 100 | $1.57 \pm 0.33 \times 10^{-6}$ |
| | 500 | $6.07 \pm 0.32 \times 10^{-4}$ |
| | 1000 | $8.14 \pm 0.20 \times 10^{-4}$ |
| | | $(2.45 \pm 0.18 \times 10^{-4})$ [b] |
| | $5.7 \times 10^6$ | $4.37 \pm 0.13 \times 10^{-3}$ |
| Bulk phase | 1000/500 | $5.05 \pm 0.31 \times 10^{-7}$ |
| | $5.7 \times 10^6$ | $4.80 \pm 0.21 \times 10^{-5}$ |

[a] Molecular number of DNA per cell. [b] Performed with a straight channel (Table S3, ESI†)

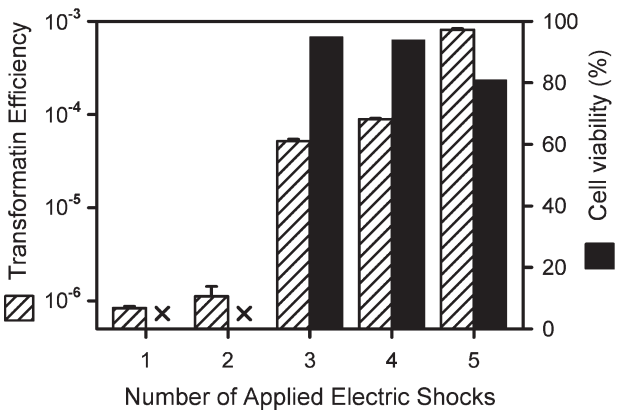



**Fig. 3** Effects of repeated electric shocks on the transformation efficiency and cell viability by on-chip electroporation, performed with a CC-400 cell-loading concentration of $1.58 \times 10^7$ cells mL$^{-1}$, a DNA/cell ratio of 1000, and a serpentine channel with different pairs of parallel electrodes. ‘×’ indicates not determined.

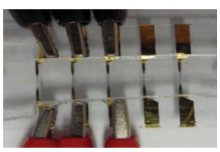
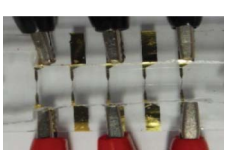
the transformation and the viability, various numbers of electrode pairs were used to arrive at a compromise between transformation and cell viability, and the results were shown in Fig. 3 (data in Table S4, ESI†). The results show that the transformation efficiency increases by an order of magnitude every time a pair of electrodes is added, until it plateaus at five pairs. In particular, there is a large jump in transformation efficiency between two and three pulses (46 times higher) but the jump from one to two pulses is only 34% and the jump from three to four pulses is only 71%. This may indicate that there is a synergistic effect between the number of electric pulses and the formed pores, resulting in enhanced entrance of DNA into the cell through the increased number of pores. They also show that the viability does not suffer much in spite of the repeated electric shocks. The high cell viability, even under repeated electric charging conditions, could be ascribed to the fast cooling, made possible by the large surface area-to-volume ratio of the droplet in the face of the Joule heating generated in the droplet. As a compromise between transformation and cell viability, five pairs of electrodes were used in the present experiments.

The effect of the time interval among multiple electric pulses on the transformation efficiency was also investigated, as shown in Table 3. Three electric pulses were applied by choosing pairs of electrodes in two ways: one is continuous pairs and the other is alternate pairs from 5 pairs of electrodes. As shown in the table, an alternative pulse process improved the transformation efficiency by a factor of $\sim$1.5, when compared with the continuous pulse process. The alternative electric pulse allowed the electroporated cell to have a twice as long recovery time from electric shock, presumably leading to improved cell viability.

### Electroporation without cell wall removal

The wild-type *C. reinhardtii* cell has a cell wall as well as a cell membrane. Unlike the cell membrane, the cell wall is not a lipid bilayer, and thus electroporation is unlikely to create pores in the wall.[30] There are pores in a native cell wall, whose size is permeable to small molecules (*e.g.* calcein) but represents a barrier to large molecules such as DNA or proteins (*e.g.* BSA).[34,35] But this cell wall can be expanded or loosened during cell growth for a transient increase in the pore size of the wall.[36] Thus, large molecules, *e.g.* DNA, can go inside cell through the

**Table 3** Effect of electric pulse interval on transformation efficiency, performed with a CC-400 cell-loading concentration of $1.58 \times 10^7$ cells $mL^{-1}$, a DNA/cell ratio of 1000, and a serpentine channel with different pairs of parallel electrodes

| Used electrodes | | Transformation efficiency |
|---|---|---|
| Continuous 3 electrodes |  | $5.19 \pm 0.27 \times 10^{-5}$ |
| Alternate 3 electrodes |  | $7.78 \pm 0.23 \times 10^{-5}$ |

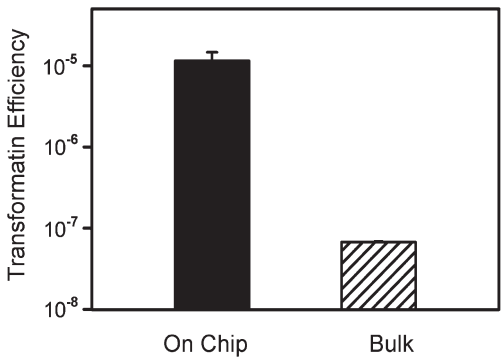



**Fig. 4** Comparative transformation efficiencies of cell wall strain, CC-124, by droplet microfluidic and bulk phase electroporation, performed with a CC-124 cell-loading concentration of $1.58 \times 10^7$ cells $mL^{-1}$, a DNA/cell ratio of 1000, and a serpentine channel with five pairs of parallel electrodes.

increased pores of the cell wall as well as the flagellar membranes. In general, it is difficult to electroporate the intact algal cell with DNA. When the wild type, cell-walled CC-124 strain of *C. reinhardtii* was used for bulk electroporation, the transformation efficiency of $6.73 \times 10^{-8}$ was generally very low, as shown in Fig. 4 (data in Table S5†), which was similar to the reported value of $1 \times 10^{-8}$–$1.2 \times 10^{-7}$, as measured under different experimental conditions.[22] Moreover, the CC-124 strain showed a $\sim$50 times lower efficiency in bulk transformation than the cell wall-less strain, CC-400, as shown in Table 2. These results indicated that the intact cell walls worked as a barrier for the entrance of DNA during the transformation. Therefore, it was often required that the cell wall was first removed by the cell wall-degrading enzyme, autolysin.[25]

In this work, however, the wild-type strain of *C. reinhardtii*, CC-124, was used for on-chip electroporation without the cell wall removal step. It was surprising that the transformation efficiency of $1.51 \times 10^{-5}$ (Fig. 4 and Table S5†) of the droplet chip process for cell wall strain was $\sim$200 times higher than that of the bulk process, although it was somewhat lower than the transformation efficiency ($8.14 \times 10^{-4}$) of the droplet chip process for cell wall-less strain. This result clearly shows that the on-chip electroporation is a very useful tool for directly transforming the cell wall strain without any additional pretreatment.

## Conclusions

A highly efficient transformation method for the algal cell was established by a cell encapsulating droplet technique using a PDMS microfluidic chip device equipped with multiple Au electrodes on a glass substrate, which was simply fabricated with no sophisticated accessories such as an expensive pulse power supply. Initially, the cell wall-less strain CC-400 of the microalgae was tested to investigate the effects of four parameters on the electroporation efficiency: ratio of DNA/algal cell, number of applied electric shocks, channel shape, and time interval between electric pulses. The best transformation efficiency of $8.14 \times 10^{-4}$ and a cell viability of 81% were obtained by electroporation using the serpentine channel with five pairs of parallel electrodes on the chip at a DNA/algal cell

ratio of 1000. This chip process showed a transformation efficiency ~1600 times higher than that ($5.05 \times 10^{-7}$) of a commercial electroporator in the bulk phase, a more than three orders of magnitude higher efficiency. Moreover, the wild-type CC-124 strain with the cell wall and membrane layers also exhibited a ~200 times higher transformation efficiency than the bulk process. In the microfluidic droplet, the rapid mass transfer effect helps facilitate the delivery of exogenous compounds into the cells, while the rapid heat transfer is favorable for high cell viability, due to quick cooling of the Joule heat generated by repeated electric charging. Therefore, the simple droplet microfluidic electroporation technique is very useful for transforming non-trivial cells with a thick wall or multiple layers. The high transformation efficiency attainable even at a low DNA/cell ratio bodes well for the control of the transgenic copy number. Finally, a still higher throughput of functional genomics could be achieved by introducing the multiple channel design into droplet microfluidics.

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