DROPLET ELECTROPORATION IN MICROFLUIDICS FOR EFFICIENT TRANSFORMATION WITH OR WITHOUT CELL WALL REMOVAL

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ABSTRACT

An efficient cell transformation method is presented that utilizes droplet electroporation on microfluidic chip. Two types of green microalgae, wall-less mutant and 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 electric equipment. The transformation efficiency via the microfluidic electroporation is shown to be more than three orders of magnitude higher for wall-less mutant, and more than two orders of magnitude higher for wild type, which has cell wall intact, than bulk phase electroporation under identical conditions. Furthermore, the microfluidic transformation is remarkably efficient even at low DNA/cell ratio, facilitating ways for controlling the transgenic copy number which is important for stability of transgene expression.

KEYWORDS

Electroporation, droplet, microalga, microfluidic, transformation.

INTRODUCTION

Microalgae, ranging in size from 1 μm to over 2 mm, have received much spotlight lately because of their potential of producing algae oil as an alternative to fossil fuel and their use for a number of industrial applications. The ability to engineer microalgae genetically and eventual manipulation of their metabolic pathway would greatly enhance their utility in science and industry. *Chlamydomonas reinhardtii*, a unicellular green microalga, is the most widely used species as a model organism.

Nuclear transformation protocols of *C. reinhardtii* are also broadly established for generating transgenic strains such as particle bombardment [1, 2], electroporation [3] and agitation with glass beads [4] or silicon carbide whiskers [5]. Among them electroporation and vortexing with glass beads are the most popular methods. In particular, electroporation achieved a maximum transformation of ~2×10^5 transformants per μg of DNA which is two orders of magnitude higher than what is obtained with the glass bead [4].

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 high electric field strength with microelectrodes. Cell handling and manipulation is easier due to the dimensions of channels and electrodes that are comparable to cell size. Furthermore, heat dissipation is fast owing to large surface area-to-volume ratio. utilization of droplet electroporation on microfluidic chip to transform microalgal cell.

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 wild-type cell with cell wall and wall-less strain of microalga, *C. reinhardtii,* were used as model cells. The high transformation efficiency made possible even at low DNA/cell ratio would eventually lead to ways to effectively control transgenic copy number.

EXPERIMENT

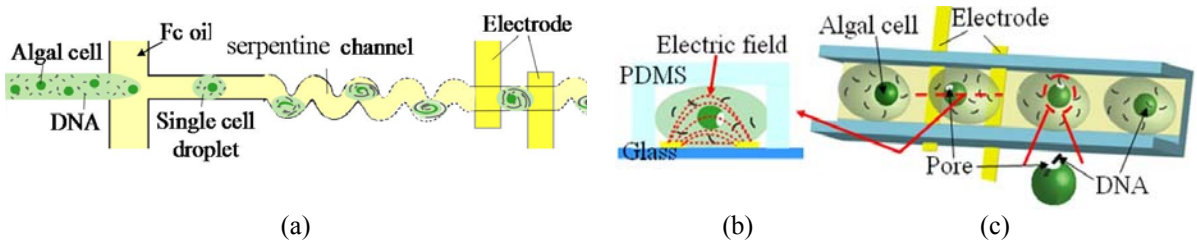

Figure 1. Principle of droplet microfludic electroporation (a) schematic diagram, (b) a droplet with cell over electrode, and (c) electroporetic process of algal cell

A highly efficient transformation method for algal cell was established by cell encapsulating droplet technique using PDMS microfluidic chip device equipped with multiple Au electrodes on glass substrate that was simply fabricated with no sophisticated accessories such as expensive pulse power supply (Fig. 1 and 2). Initially, the wall-less strain CC-400 of microalgae was tested to investigate the effects of four parameters on the electroporation efficiency: ratio of DNA/algal cell (Table 1), number of applied electric shock (Table 2), channel shape, and time

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interval between electric pulses (Table 3). The best transformation efficiency of 8.14×10^{-4} and cell viability of 81% were obtained by droplet electroporation in microfluidic using the serpentine channel with five pairs of parallel electrodes on chip at a DNA/algal cell ratio of 1000. This chip process showed a transformation efficiency ~1600 times higher than that (5.05×10^{-7}) of commercial electroporator in bulk phase, a more than three orders of magnitude higher efficiency. Moreover, the wild-type CC-124 strain having the cell wall and membrane layers also exhibited ~200 times higher transformation efficiency than the bulk process (Table 3).

The droplet microfluidic electroporation must be useful for efficient transformation of algal cells as well as other microorganisms that are difficult to transform. Furthermore it facilitates ways for controlling the transgenic copy number which is important for stability of transgene expression.

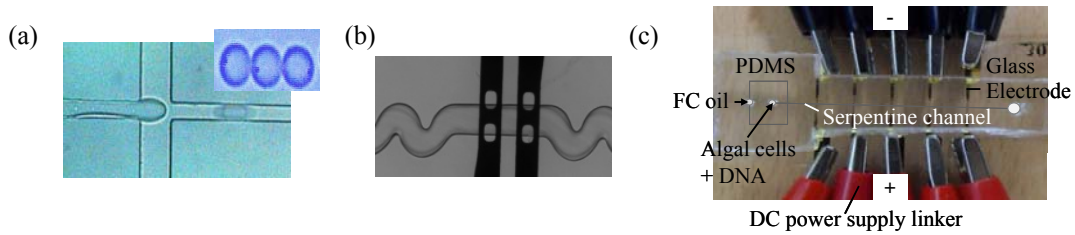

Figure 2. Algal cell electroporation on microdludic chip: optical image of (a) droplet generation and single cell encapsulated in a droplet, (b) serpentine channel and electrode, and (c) whole electroporation chip system. The scale bar is 50 μm

Table 1. Effect of DNA/algal cell ratio and electroporation techniques on transformation efficiency. Transformation was performed with 1.58×10^{7} cells/mL of CC-400 cell-loading concentration, and serpentine channel with five pairs of paralleled electrodes.

Transformation	*DNA /algal cell	Transformation efficiency
On chip	100	$1.57\pm0.33\times10^{-6}$
	500	$6.07\pm0.32\times10^{-4}$
	1000	$8.14\pm0.20\times10^{-4}$ $^{+}(2.45\pm0.18\times10^{-4})$
	5.7×10^{6}	$4.37\pm0.13\times10^{-3}$
Bulk phase	1000 / 500	$5.05\pm0.31\times10^{-7}$
	5.7×10^{6}	$4.80\pm0.21\times10^{-5}$

*molecular number of DNA per cell, $^{+}$ performed with straight channel

Table 2. Effect of repeated electric shocks on the transformation efficiecy and cell viability by on-chip electroporation. Transformation was performed with 1.58×10^{7} cells/mL of CC-400 cell-loading concentration, 1000 DNA/cell ratio, and serpentine channel with different pairs of parallel electrodes.

Electrode pairs	Transformation efficiency	Cell viability (%)
1	$8.37\pm0.33\times10^{-7}$	-
2	$1.12\pm0.31\times10^{-6}$	-
3	$5.19\pm0.27\times10^{-5}$	95
4	$8.88\pm0.21\times10^{-5}$	94
5	$8.14\pm0.20\times10^{-4}$	81

Table 3. Comparison of transformation efficiency between droplet microfluidic and bulk phase electroporation of cell walled strain, CC-124. Transformation was performed with 1.58×10^{7} cells/mL of CC-124 cell-loading concentration, 1000 DNA/cell ratio, and serpentine channel with five pairs of parallel electrodes.

Electroporation mehtod	Ratio of DNA to cell	Transformation Efficiency
On chip	1000	$1.51\pm0.32\times10^{-5}$
Bulk phase	1000	$6.73\pm0.35\times10^{-8}$

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