Biochemical Engineering Journal 122 (2017) 133–140

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Transfection of Jurkat T cells by droplet electroporation

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ARTICLE INFO

Article history:
Received 11 September 2016
Received in revised form 15 March 2017
Accepted 16 March 2017
Available online 18 March 2017

Keywords:
Transfection
Cancer research
Droplet
Electroporation

ABSTRACT

A droplet electroporation system has been successfully demonstrated for transfection of Jurkat T cell with much higher transfection efficiency (66%) than that of conventional system (11.4%) with the advantages of superior cell viability, comparable throughput, and user-friendly interface. Because the productivity of the proposed system is much greater than previous microfluidic systems, flow cytometry is used for the analysis of transfection efficiency. The high transfection efficiency and cell viability of the droplet electroporation system is mainly attributed to small size which requires lower voltage and provides concentrated environment. The successful transfection of Jurkat cell using the droplet electroporation system broadens the applicability of the proposed technology to medical field. The implication of the present work and the future development direction for a fully automated cell engineering platform are discussed.

1. Introduction

Jurkat T cells are an immortalized line of T lymphocyte cells that are used to study acute T cell leukaemia and T cell signaling [1]. They are also useful in many biomedical fields because of their ability to produce interleukin 2 and their use to determine the mechanism of differential susceptibility of cancers to drugs [2]. However, they are notorious for their low efficiency of transfection comparing with usual adherent cell lines [3].

Among conventional non-viral DNA delivery systems, electroporation (EP) is one of the most widely used technologies [4–6]. EP is a physical process that uses high-voltage electric pulses to transiently permeabilize cell membrane, thus making pathways for cellular uptake of exogenous materials. Not only as an efficient gene transfection method *in vitro*, EP has also been used in numerous *in vivo* medical applications such as electroporative delivery of chemotherapeutic drugs into tumor cells [4,6,7], gene therapy [4,8] and DNA vaccination [9,10].

Although conventional bench-top EP system has been used more than two decades, it has some limitations due to the use of relatively high voltages [11]. To overcome these limitations, microfluidic approaches have been tried [8,11–20]. In microscale EP system, applied voltages can be much reduced, which increases the cell viability and transfection efficiency [21]. The microfluidic environment also offers increased precision and control for gene delivery [12,14]. However, the current microfluidic EP systems also have several limitations such as low productivity [11,15] and the lack of ease-of-use interface (such as a user-friendly pipette) for clinical standards and applications [11]. Recently, a digital microfluidic EP (digital EP) system based on the direct charging and subsequent electrophoresis of a charged droplet (ECD) [22–32] has been proposed to overcome the limitations of both the conventional and microfluidic EP systems [33] (consult the supplemental material for comparison with various EP methods.). Thanks to the unique features of the digital EP system, the proposed system accomplished one order of magnitude higher transgene expression over the conventional EP system in the transformation of wild-type microalgae without cell wall removal [33]. However, there is no report for the transfection of mammalian cells using the proposed digital EP system yet. Furthermore, there was insufficient description on the background of the possibility for future cell engineering platform more in detail.

Here, we present the successful performance of an ECD based droplet EP system for transfection of Jurkat T cell. By investigating effects of the EP conditions such as pulse voltage and number of pulses on the viability and transfection efficiency in comparison with a conventional EP system, efficient EP condition for Jurkat cell transfection has been found. Furthermore, about five times higher transfection efficiency than that of conventional system as well as reported literatures has been accomplished. The implications of the present work not only in an efficient transfection tool but also in an automated cell culture and engineering platform are discussed.

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http://dx.doi.org/10.1016/j.bej.2017.03.010

2. Materials and methods

2.1. Droplet EP system preparation

A droplet EP system based on ECD was prepared for EP process and the manipulation of droplets as shown in Fig. 1. Basic design concept and structures (using pin header sockets and pin headers with 2.54 mm pitch and acrylic structures) are similar to that of the previous work [33] (the details of fabrication procedures and circuit connections are described in Supplemental Material.). The prepared fluidic component was filled with silicone oil (DC200F, 6 cSt) and connected to the connecting base for the monitoring of current flow by an electrometer (Keithley 6514). A syringe needle coated with carbon paste (ELCOAT CX-12, CANS) was aligned with one of the bottom electrodes of the fluidic component as an electrode for EP process (EP needle). Using a micropipette, a 2 μL DNA/cell suspension droplet was dispensed between the needle and the bottom electrode (with 1 mm gap). A square pulse voltage (32–64 V) with different numbers of pulses n (4–16 times with 50 ms duration and 100 ms break between pulses) was applied for EP. For the parametric study of EP conditions, five cases (three cases of 32, 48, 64 V with n = 8 and two cases of n = 4 and 16 with 48 V) were considered. After the EP process, the processed droplets were gathered into the syringe attached to the EP needle right after the EP by a manual suction. To prevent cross contamination between different experiments, the EP needle and the syringe were replaced in each experiment.

2.2. Cells and plasmid DNA preparation

Jurkat cells (a human T lymphoma cell line derived from an acute T cell leukaemia) were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco/Invitrogen) supplemented with 10% fetal bovine serum (Hyclone), 10 mM Hepes, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Prior to use, cells were centrifuged and diluted with fresh medium and placed in a 6 well plate with the total number of 1×10^6 cells in each well (1×10^6 cells were used in each experiment.).

The plasmid DNA pEGFP-N1 expressing the enhanced green fluorescent protein (GFP) were purchased from ClonTech. E. coli bacteria were made to express CMV-GFP and the transformed bacteria were grown in Luria-Bertani (LB) broth (0.5–1.0 L) of supplemented with 100 mg/mL kanamycin. Using a Plasmid Mini Kit from QIAgen (QIAGEN Cat. No. 74903), the plasmid DNA was purified and diluted in sterilized water. The purity of DNA was confirmed by 1% agarose gel electrophoresis after ethidium bromide staining. The DNA concentration was measured by a spectrophotometer (NanoDrop 2000c, Thermo) at 260 nm. All through the experiments, 1 μg DNA (approximate number of DNA copies is 1.96×10^{11}) was used in each experimental condition to maintain DNA molecules/cell concentration ratio (1.96×10^5 DNA molecules/cell) constant.

2.3. Conventional and droplet EP procedures

Gene Pulser Xcell (Bio-Rad) was used as a conventional EP system. We followed the EP protocols of previous work [33]. For efficient EP process, serum was removed from the cell suspension and the volume of DNA/cell suspension was 500 μL and processed in a 4 mm electrode gap cuvette. Once placed in the holder of the Bio-Rad EP apparatus, an equivalent electric shock (192 V/4 mm) to the corresponding droplet EP condition (48 V/mm) was applied. After the EP process, we used the same post processing as previous for the evaluation of EP performance (24 h CO_2 incubator culture in 1 mL culture medium containing serum).

For the droplet EP transfection, the cultured cell suspension was transferred in e-tube and centrifuged for 1 min at 97.24 RCF (1200 rpm, rotor GAM-1.5-12). Most of culture medium was removed leaving approximately 9 μL behind. The prepared 1 μg DNA containing the gene of pEGFP-N1 (1.5 μL) was added to this e-tube making 10.5 μL DNA/cell suspension. The mixed DNA/cell suspension was incubated on ice for 5 min before the droplet EP experiment. The DNA/cell suspension was provided as a small droplet (approximately 2 μL) using a micropipette to the 1 mm gap between the EP needle and one of the bottom electrodes of the fluidic component. The EP electric shock was applied to this 2 μL droplet and after the EP, the droplet was sucked into the syringe attached to the EP needle by manual suction. In order to obtain confocal microscope images and FACS analysis results, we needed about a million cells. Contrary to a conventional EP system in which a single shot process is enough for getting a million cells, in the droplet EP system, repeated EP processes are needed. Therefore, the droplet EP process was repeated 5 times using five individual 2 μL samples (it took about 3 min for the whole 10 μL sample processing) and the gathered 10 μL DNA/cell suspension in the syringe was transferred to another e-tube. Because the gathered DNA/cell suspension sample contained silicone oil, the oil was removed using a glass suction tip connecting to a suction pump. The remaining 10 μL DNA/cell suspension was directly added to a pre-warmed 1 mL medium for 24 h incubation in a CO_2 incubator.

In conventional EP process, serum removal was required to increase transfection efficiency because serum contains antibodies, antigens, hormones, and any exogenous substances that can interfere with the delivery of external DNA into cells. However, because of higher efficiency of the proposed system, we don't have to remove serum. Furthermore, because cells are handled with original cell culture medium we also don't have to exchange medium after electroporation. There will be some changes in the cell suspension medium after EP process but because handled cell suspension volume is small (10.5 μL) compared with cell culture medium in well for 1-day culture (1 mL), we don't have to worry about these changes. Therefore, the procedure of the droplet EP method became simpler due to no need of serum removal as pre-processing and the replacement of culture medium as post-processing (the flowchart for the entire experimental procedure is described in Supplemental material.). For replication, all the experiments were duplicated for each experimental condition at different days.

For the control group, we have two choices: one experiences the same EP conditions as the experimental group but in the absence of DNA and the other having the same number of cells and DNA as the experimental group but in the absence of EP operation. We chose the latter for the present work because we use various EP conditions and it will be too complicated if we choose the former as the control group (FACS setting was adjusted to exclude auto-fluorescence from dead cells as in the previous work [33]). The control group was designed to provide a basis for the evaluation of viability and transformation efficiency. The same number of cells (1×10^6) as the experimental groups were prepared in a 1 mL culture medium with 1 μg DNA and incubated in a CO_2 incubator for 24 h. The control group represents the natural uptake of external DNA into cells without any electric field effect.

2.4. Evaluation of transfection efficiency and cell viability

After 24 h incubation, the transfection efficiency and cell viability of each sample were evaluated by a FACS (Gallios A94303, BECKMAN COULTER). Cells were harvested by centrifuging 5 min at 152 RCF (1500 rpm, rotor GAM-1.5-12), 4 °C. After removing culture medium, pelleted cells were re-suspended in 500 μL phosphate buffered saline (PBS) and transferred into a FACS tube and propidium iodide (PI, 1 μg/mL) was added to the FACS tube for the