Roles of electric field and current in composite gene transfection with plasma and pulsed electric field

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Abstract: As a new gene transfection technology, we propose an improved gene transfection using the plasma and electric pulse in cell culture medium. This method achieved a high transfection efficiency over 50% and a high cell viability over 70% and was shown to be promising for high quality gene transfection. By the experiments to isolate the transfection effects of electric field and current, optimization of electric current might be key for highly efficient gene transfection with high cell viability.

Keywords: In liquid plasma, pulse electric field, gene transfer

1. Introduction

Molecular transfection technology is the process of intentionally introducing extracellular molecules such as drugs, proteins, and genes into living cells. There is a big demand of transfection technology in various fields such as biological and medical field because it is used for cancer therapy, genetic modification and so on. Conventional transfection methods such as lipofection, electroporation, and viral vector methods have their advantages, while they have some problems such as low transfection efficiency, low cell viability, and inability to introduce genes into specific cells. As an alternative approach to these methods, gene transfer using arc plasma was proposed in 2005 [1], having advantages such as high efficiency and ease of use and some problems such as low cell viability. In the past two decades, many research groups have reported gene transfer methods using various atmospheric pressure plasmas (APPs) in contact with liquids [2-5]. However, the general APP is generated in a gas phase, and it is considered that transfection key factor(s) generated by APP was supplied to cells through a liquid phase. Since cells are usually at deep region in liquid, transfection effect by APP is likely to be ineffective at deep part in solution.

Recently, our group has reported that plasma generated in saline also could transfer molecules into living cells [6]. This method can easily treat cells at deep part in solution. In this study, an improved gene transfection using plasma and electric pulse in cell culture medium is newly proposed. Specifically, effects of electric field and current on gene transfection efficiency and cell viability were intensively investigated.

2. Experiment setup

Plasma was generated by applying a pulse voltage (peak voltage V_{in} = 1.0 ~ 1.3 kV, pulse width T_p = 100 μs, applying frequency f = 1Hz) to coaxial type lab-built pin electrode which has a curvature radius of less than 100 μm as shown in Fig. 1(a). This enabled stable discharge in conducting solutions [6, 7]. To increase the efficiency of gene transfer, not only plasma in liquid but also pulsed electric field was employed. A pulse power source [Fig. 1 (b)] independent of the one for plasma generation was used to apply the pulsed electric field. The pulse width was

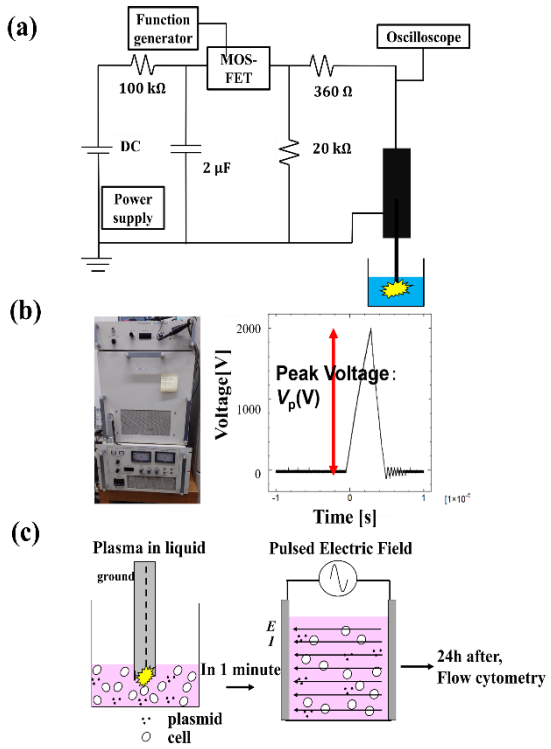

Fig. 1. (a) Circuit diagram for plasma generation in liquid. (b) Photo of a power supply for pulsed electric field and a typical voltage waveform of applied electric pulse. (c) Experimental procedure of composite gene transfection with plasma and pulsed electric field.

Table 1. Composition of EPB.

Material	Concentration (mM)
Sucrose	285
KCl	1
$MgCl_2$	0.7
HEPES	10
NaOH	Proper quantity(Adjusted to pH 7.4)

approximately several μsec, extremely short compared to that (~ msec) of the conventional electroporation, could lead to less cell damage. Adherent cells (human breast cancer cell, MCF-7) suspended in serum-reduced cell culture medium, Opti-MEM (31985070, Thermo Fisher Scientific) or lab-made electroporation buffer (EPB) as shown in Table 1 [8] were treated in this study [Fig. 1(c)]. Plasmid DNA (pAc-GFP1C1) which encodes green fluorescent protein (GFP) was added to the solutions beforehand. In order to change the applied electric field with maintaining the applied electric current, the conductivity of the EPB was adjusted by changing the ratio of KCl to sucrose. Treated cells were incubated for 24 - 48 hours (37°C, 5% CO_2) and the transfection efficiency and the cell viability were evaluated by a fluorescence microscope and flow cytometry.

3. Results and discussion

Figure 2 shows a typical result of composite gene transfection with plasma and pulsed electric field in Opti-MEM. Transfection efficiency was 46.3% for the in-liquid plasma alone, 16.7% for the pulsed electric field alone, and 50.7% for the combined method. Thus, the combined method seemed to be better than each single treatment in the transfection efficiency. However, the cell density 24 - 48 hours after the combined method slightly decreased compared to single treatment, indicating a minor increase in cell damage.

Next, in the pulsed electric field alone, we investigated the effects of electric field and current on gene transfection efficiency and cell viability. Here, the applied peak current (I_p) is kept constant (1 ± 0.2 A) and the peak voltage (V_p) is varied from 50 to 560 V using EPB with various electric conductivities (1.11 to 7.64 mS/cm). Figure 3 shows the transfection efficiency was hardly improved with varying V_p, while cell viability was drastically decreased at $V_p = 560\,V$. This indicates that both of high efficiency and high cell viability could not be achieved with this constant electric current ($I_\mathrm{p} = 1$ A). As shown in Fig. 2(c), the gene transfection of 16.7% was observed in the pulsed electric field alone with $I_\mathrm{p} \approx 7$ A and $V_p \approx 300$ V. Therefore, optimization of I_p rather than V_p might be key for more efficient transfection.

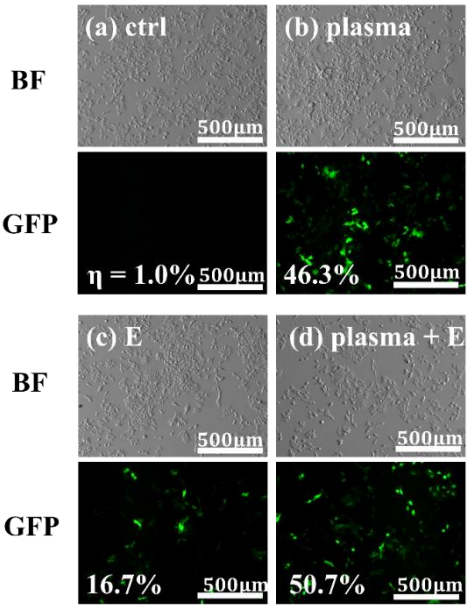

Fig. 2. Bright field (BF) and GFP fluorescent images of cells treated by (a) control, (b) the in-liquid plasma alone, (c) the pulsed electric field alone, and (d) the composite method (plasma + E) in Opti-MEM.

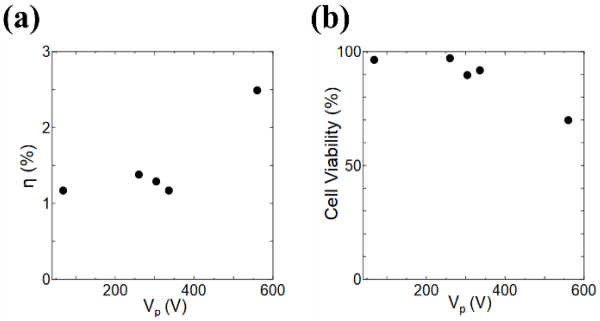

Fig. 3. (a) gene transfection efficiency (η) and cell viability as a function of the applied peak voltage (V_p) with the applied peak current (I_p) is kept constant (1 ± 0.2 A).

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