GENE TRANSFER BY CIRCULATING PLASMA BUBBLE FLOW

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ABSTRACT

We have succeeded in injection of plasmid to adherent cells which are suspended in the plasma-bubbles laden circulation flow in a chamber. High-speed plasma-bubbles are generated by glass electrode and the air-liquid interface has a stiction force which draws the gene (plasmid) and stick to the air-liquid interface. The circulating flow increased the chance for cells to contact air-liquid interface of bubbles which enclosed plasma or reactive gas. Finally, the high reactive interface enables gene transfer to cells efficiently. This technology of two-dimensional microfluidic chip contributes an option to the high-throughput gene transfer.

INTRODUCTION

Recent progress of the gene transfer provides many findings of mechanism of cell, and many researchers analyze and control the phenomena in the cell based on the information [1]. However, the limited methods were available for the high efficient injection, especially hard materials such as plant cells or cyanobacteria. For example, electroporation method has advantages for gene transfer to a number of cells at a time. However, the survival rate after the injection sometimes reduced remarkably, which is due to the lack of control of applied voltage [2]. It is expected to develop novel injection methods which are minimally-invasive, high efficient and high-throughput. We propose electrically-induced bubble injection device which can eject plasma and high-speed bubble. This plasma bubble injector can dispense bubbles enclosing plasma or reactive gas which enables to gene transfer to suspended cells indirectly by contacting cell and air-liquid interface of bubbles. The suspension of plasma bubbles is expected to provide high throughput gene transfer within a limited-time.

CONCEPT

Figure 1 shows the concept of high-throughput gene injection by using combination of bubbles and plasma. One of the functions of plasma is high-reactivity and minimally invasive perforation of cells with diameter of less than 1μm [2]. This is due to the effect of radical species of plasma. In addition, the bubble itself functions in high transportation ability because of the adsorption effect of air-liquid charged interface. The synergy effects of both functions provide high throughput gene transfer, especially for high concentration of cells.

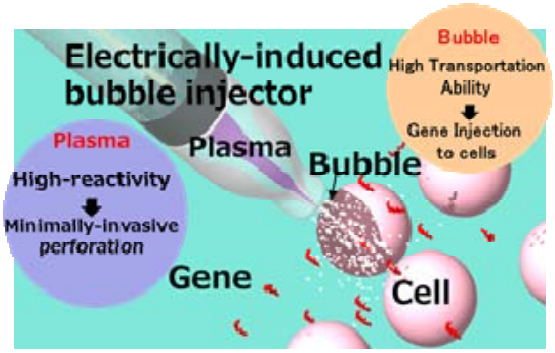

Figure 1: High-throughput gene injection by using combination of bubble and plasma

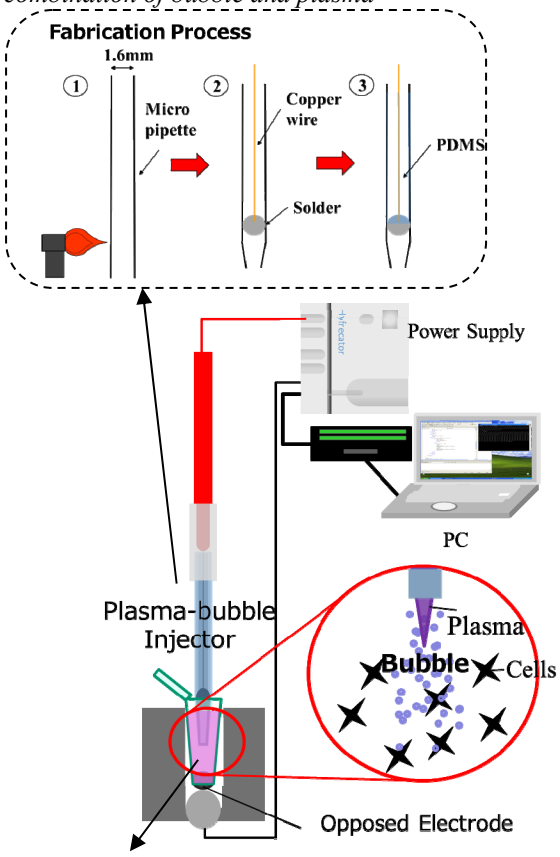

Figure 2: Fabrication process of plasma-bubble Injector and experimental set-up (Modified sampling tube method)

EXPERIMENTAL PROCEDURES 1

Injection Using Modified Micro-tube with Electrode

Figure 2 shows the fabrication process of plasma-bubble injector and experimental set-up. HeLa cells are used as adherent cells, and the cell-suspension in medium was made by trypsin treatment. First set-up used a 1.5-ml snap-cap tube equipped with an opposed electrode at the bottom of tube (the modified micro-tube with electrode). The plasmid pEGFP-C3 carrying the gene for GFP was used for the plasma-bubble injection. The concentration of pEGFP-C3 was set to 50 μg/ml, and the cell density was set to 5×10^6 cells/ml.

EXPERIMENTAL RESULTS 1

Injection by modified micro-tube with electrode

The plasma-bubble is successfully injected the plasmid DNA into the cells. The injection rate and survival rate of the cells were evaluated. Figures 3 and 4 show the experimental results evaluated by input power and exposure time. It seems that the input power affects the survival rate, the exposure time affects the injection rate. To overcome the efficiency of electroporation method, the improvement of the injection device is required to increase chances to contact between cells and radical species.

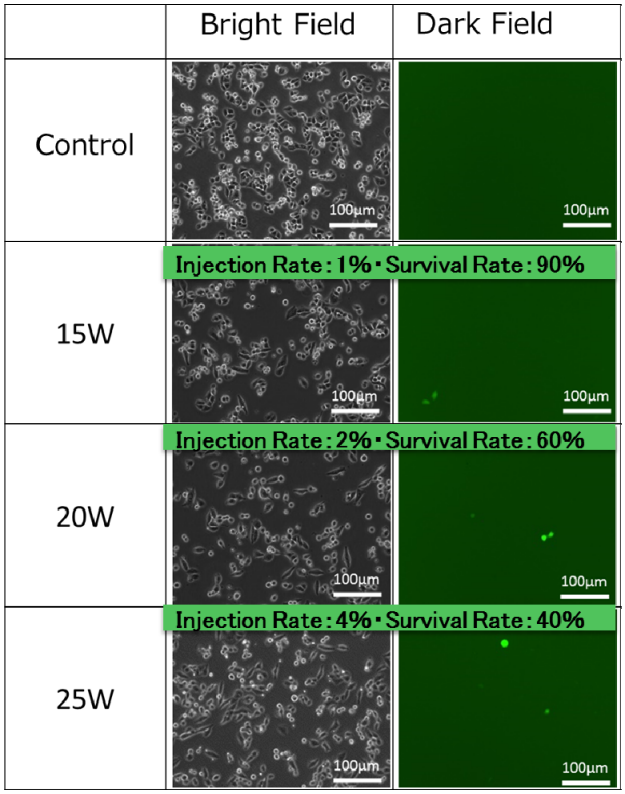

Figure 3: Evaluation of Injection & Survival Rate as a function of input power for HeLa cells (exposure time = 1 sec.).

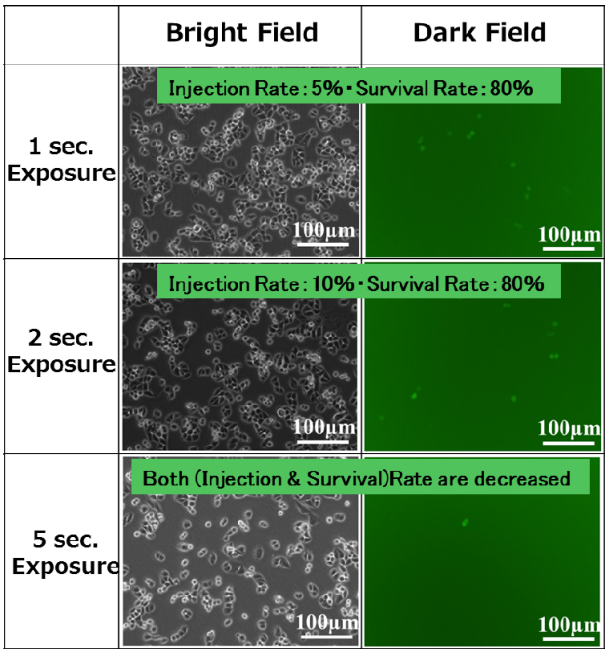

Figure 4: Evaluation of Injection & Survival Rate as a function of exposure time for HeLa cells (1 pulse = 0.04 ms, pulse interval= 100 ms).

EXPERIMENTAL PROCEDURES 2

Circulating Plasma Bubbles Chip with Rotor

To increase the injection rate, we have revised the design of injection device that has the circulating two-dimensional microfluidic chip as shown in Figure 5. The chip has a rotor which is rotating by the jet flow produced by plasma-bubble injector. The experimental set-up for the revised-device is shown in Figure 6. The rotor and circulating plasma bubble-chip is fabricated by 3D printer, and plasma-bubble injector is connected to the inlet part of the chip.

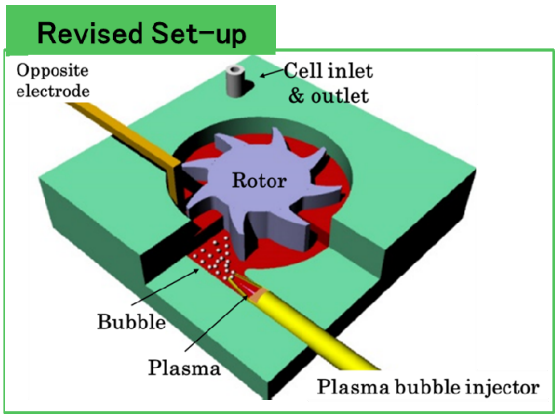

Figure 5: Revised concept of gene transfer with circulating plasma-bubble chip for longer exposure time

【Injection Target】 Cell: HeLa cells
(Suspending in medium after trypsin treatment)
【Injection Material】 Gene : pCX-EGFP

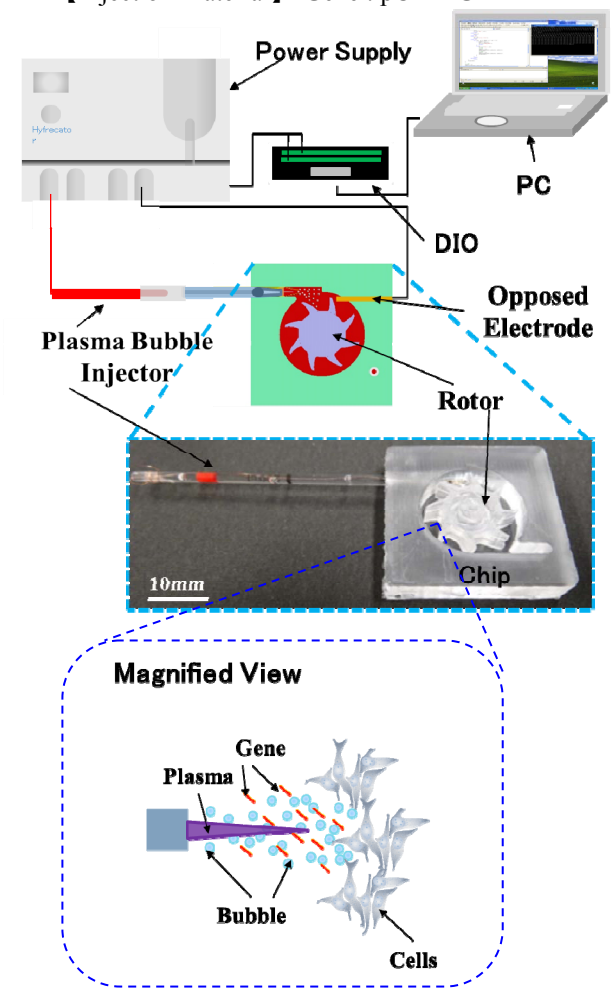

Figure 6: Experimental set-up for circulating plasma-bubble chip for gene injection chip for higher injection rate

EXPERIMENTAL RESULTS 2

Circulating Plasma Bubbles Chip with Rotor

Figure 7 shows the experimental result of the transfection of pCX-EGFP into HeLa cells using circulation chip with rotor. It was confirmed that the injection rate increased along with the increases of the exposure time. Compared to the conventional set-up (Figure 2), it was confirmed that the injection rate successfully increased by 25% for the condition of 5 sec. exposure time for the applied power of 15W. This design of the chip seems to accelerate the contact between the cells and radical species.

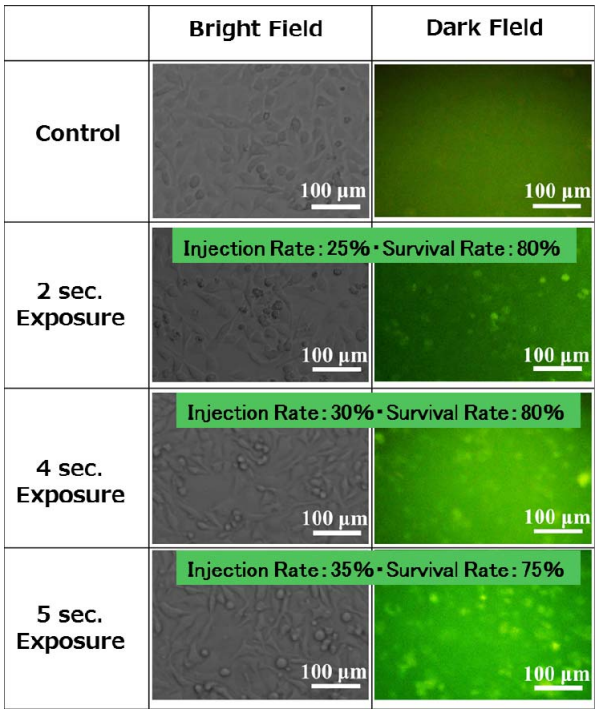

Figure 7: Evaluation of Injection & Survival Rate as a function of exposure time using plasma bubble circulation chip for HeLa cells (1 pulse = 0.04 ms, pulse interval= 100 ms).

EXPERIMENTAL PROCEDURES 3

Circulating Plasma Bubbles Chip without Rotor

After the injection experiment using rotor chip, some of the cells are stuck under the rotor, and moreover the rotor did not provide steady circulation in the chamber of the micro-fluidic chip. This is likely caused by the pressure drag of the rotor. Therefore, we have revised the chip without using rotor as shown in Figure 8.

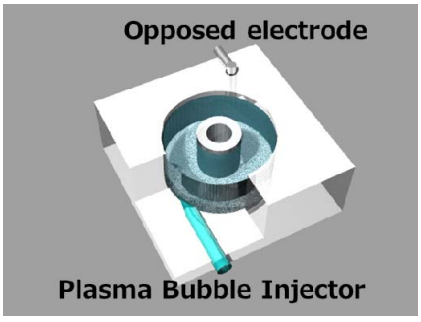

Figure 8: Revised design circulation chip without using rotor.

This type of the chip transmits the jet flow to the circulation flow in the chamber more efficiently. This type of the chip is expected to increase the chance which the cells contact to radical species in the chamber.

This time the injection target is changed from HeLa cells to yeast cells to show the advantage points over the electroporation injection technology. Generally, it is very difficult to injection reagent or genes to yeast cells by using physical injection method. However, this plasma-induced injection method may have some possibility to inject efficiently with the aid of plasma induced radical species.

EXPERIMENTAL RESULTS 3

Circulating Plasma Bubbles Chip without Rotor

Figure 9 shows the result of fluorescent reagent (Alexa Fluor 594 (2 [mg/mL]) injection by using the chip shown in Figure 8. The molecular size of fluorescent reagent is 10,000 MW with dextran to prevent penetration and dying by small molecular size. The cells are washed sufficiently after the injection experiment by centrifugation.

It was confirmed that the injection rate remarkably increased with the increased exposure time of plasma-bubbles.

The cells in the bright field picture of 35 seconds exposure time seems similar condition with that for the 6 seconds exposure. Further studies are required to evaluate the survival rate in the future.

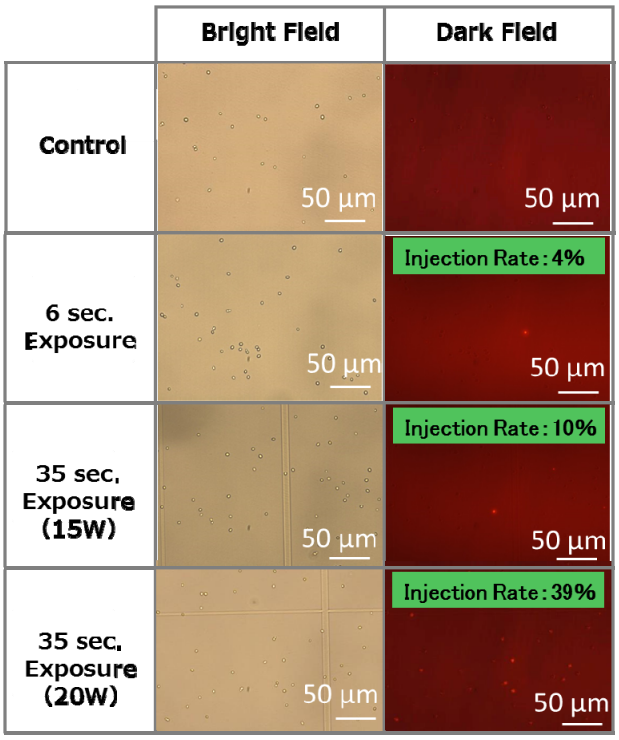

Figure 9: Evaluation of Injection & Survival Rate as a function of exposure time for yeast cells (1 pulse = 0.04 ms, Pulse interval= 500 ms).

CONCLUSIONS

For the present study, a new gene injection method using plasma and bubbles is proposed. This technology is simple and low in cost, and contributes to the high-throughput and automated gene transfer processes with a high efficiency. One of the advantage points over the other injection technology is that this technology has a potential to injection to the cells with hard shell with minimally-invasiveness.

This technology has a potential to contribute to the hard material such as yeast cells or cyanobacteria into which it is difficult to inject reagent or gene. This method can contribute to wide range of research fields such as gene therapy and direct gene injection to biological materials of various hardness.

ACKNOWLEDGEMENTS

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