Highly-Efficient Transfection of Genes and Macromolecules Using Atmospheric Pressure Plasma

大気圧プラズマを用いた遺伝子・高分子の高効率導入

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To develop a highly efficient and minimally invasive gene transfection method, the enhancement of cell membrane permeability after direct plasma irradiation under various conditions is evaluated from fluorescence dyes intensity using a confocal laser scanning microscope. As a result, it is found that global or local transfer of the fluorescent dyes and siRNA can be controlled depending on cell states during plasma irradiation. Under same plasma conditions, the cell proliferation is normal and obvious cell damage is not detected.

1. Introduction

Gene transfection, which is the process of deliberately introducing nucleic acids into cells, is expected to play an important role in molecular biology and the medical treatment such as gene therapy, the siRNA-mediated technique to suppress gene expression [1], and creation of induced pluripotent stem (iPS) cells [2]. However, the conventional gene transfection methods, such as lipofection [3], electroporation [4], and the viral vector method, have some problems that the transfection efficiency is not so high, the cell viability is low, and the genes cannot be transferred into some specific lipid cells. On the other hand, recently, gene transfer using discharge plasma has attracted attention [5, 6]. However, the mechanism of the gene transfection using the plasma is not clarified. Therefore, we try to use controlled non-equilibrium atmospheric pressure plasma jet (APPJ) and investigate the mechanism toward developing highly-efficient and minimally-invasive transfection [7].

2. Experimental Apparatus

Figure 1 shows schematic illustrations of the experimental setup for gene introduction into (a) the suspended cells and (b) the adherent cells using APPJ. Helium is served as the source gas, with its flow rate (f) through the dielectric tube and is regulated by a mass flow controller (MFC), typically, f = 3 slm. When the high-voltage ($V_{p\text{-}p}$) power supply (with a frequency of 10 kHz) of this system is turned on, dielectric barrier discharge (DBD) plasma is generated and flows out from the nozzle of the quartz glass tube (2-mm inner diameter), irradiating a 100-μL solution containing living cells and the transferred materials (c μM) for a controlled time (t_i). The typical voltages are (a) $V_{p\text{-}p}$ = 11.6 kV, (b) $V_{p\text{-}p}$ = 8.7 kV, and distances

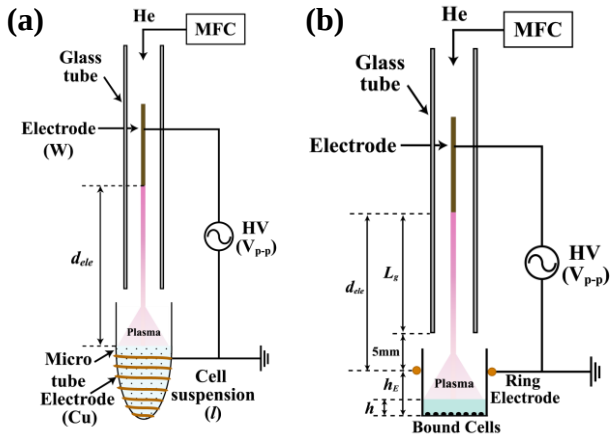

Fig.1: Schematic illustrations of the experimental setup for gene introduction into (a) the suspended cells and (b) the adherent cells using APPJ.

are (a) d_{ele} = 60 mm, (b) d_{ele} = 26 mm, h_E = 6 mm, h = 1.3 mm.

In this experiment, we used the fluorescent dye YOYO-1 (green) or TOTO-3 (red) besides actual genes as the transferred material to quickly investigate the effect of plasma irradiation on cell membrane permeability. In addition, small interfering RNA labeled by fluorescent dye Cy5 (siRNA-Cy5) is used as the actual gene. In both cases, when the transferred materials are inside cell membrane, the cell exhibits strong fluorescence.

3. Experimental Results and Discussion

Figure 2 shows flow cytometry histogram of the

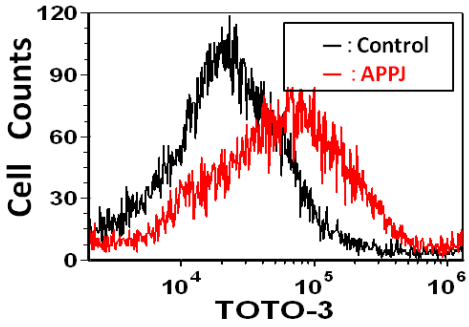

Fig.2: Flow cytometry histogram of the TOTO-3 transferred cells detection by plasma irradiation for t_i = 1s, c = 5 μM, and l = 100 μl.

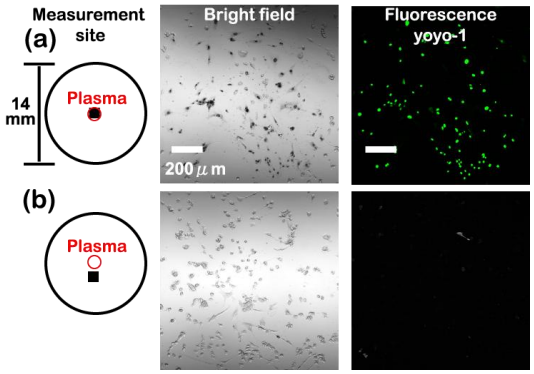

Fig.3: Bright field and green fluorescence images of the adherent cells (a) inside and (b) outside the plasma-irradiated region.

TOTO-3 transferred cells (suspended human breast cancer cells [MCF-7]) detection by plasma irradiation. The cells after plasma irradiation globally exhibits stronger fluorescence compared with control (not irradiation), which means that extracellular TOTO-3 is transferred inside cell membrane.

Figure 3 presents bright field and fluorescence images of the adherent mouse fibroblast cells [3T3L1] (a) inside and (b) outside the plasma-irradiated region. Effective region of the plasma is limited to the plasma-irradiated region. This result indicates the electric field effects of charged particles are the most critical for transfection. Only in the plasma-irradiated region, more than 80% of the transfected cells were observed. Moreover, cell proliferation after plasma irradiation under these conditions remains intact.

Thus, as shown in Fig.2 and Fig.3, depending on a adhesive or floating state, we can select global or local treatment.

Figure 4 shows green (green fluorescent protein; GFP) and red (Cy5) fluorescence, and bright field images of (a) a non-treated and (b) plasma-treated suspended cell for t_i = 1s, c = 2.5 μM, and l = 100 μl. Target cell is MCF-7 in a floating state that

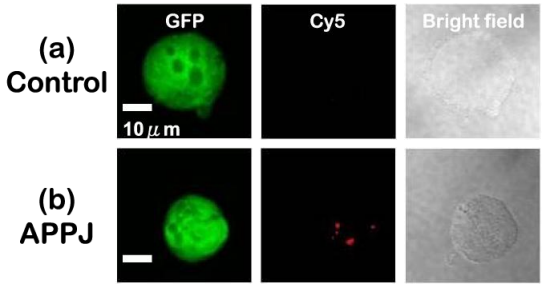

Fig.4: Green (GFP) and red (Cy5) fluorescence, and bright field images of (a) a non-treated and (b) plasma-treated suspended cell for t_i = 1s, c = 2.5 μM, and l = 100 μl.

stably expresses enhanced GFP (MCF7-eGFP). The plasma-irradiated cell exhibits nearly equal green fluorescence compared with control (non-irradiated), which means that intracellular proteins are not damaged. On the other hand, stronger red fluorescence is observed in a part of the plasma-irradiated cell, which suggests siRNA-Cy5 is transferred into the cell. Therefore, this result shows that actual nucleic acids can be transferred using plasma irradiation just like fluorescent dyes.

4. Conclusion

We have investigated enhancement of cell membrane permeability using direct APPJ irradiation toward developing highly-efficient and minimally-invasive transfection. As a result, depending on ways of plasma irradiation, we could successfully realize global or local transfer of the fluorescent dyes and siRNA into living cells with no obvious cellular damage.

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