17p-S10-9

プラズマ制御照射による生細胞への DNA および RNA の導入

Transfection of DNA and RNA into Living-Cell by Controlled Plasma Irradiation

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Non-equilibrium plasmas irradiated to the living-cell suspended solution are investigated for medical applications such as gene transfection, which is expected to play an important role in molecular biology, gene therapy, and creation of induced pluripotent stem (iPS) cells. To develope highly-efficient and minimally-invasive gene transfection, we have used controlled atmospheric pressure non-equilibrium plasma and investigated the transfection mechanism using fluorescent dye YOYO-1 [1]. Here, we try to transfer actual genes such as DNA and RNA into the living-cell by precisely controlled plasma irradiation.

The atmospheric pressure plasma is generated using low frequency (LF) (frequency: 10 kHz, voltage: $V_{p\text{-}p}$ kV) with He gas flow, which is irradiated to the living-cell suspended solution mixed with genes. In this experiment, siRNA (21bp) and plasmid DNA (4.7kpb) are used as the actual genes. Here, to identify the transfection by the fluorescence image, the siRNA are functionalized by fluorescent dye Cy5 and the plasmid DNA codes for green fluorescent protein (GFP).

Figure 1 shows bright field and fluorescence images of (a) human breast cancer cell (MCF-7) with siRNA functionalized by red fluorescent dye (Cy5) and (b) mouse fibroblast cell (3T3L1) with plasmid DNA coding for GFP after plasma irradiation to the cell with precise control. The red and green fluorescence is observed inside the cell, which means the actual genes such as siRNA and plasmid DNA can be transferred into the MCF-7 and 3T3L1, respectively.

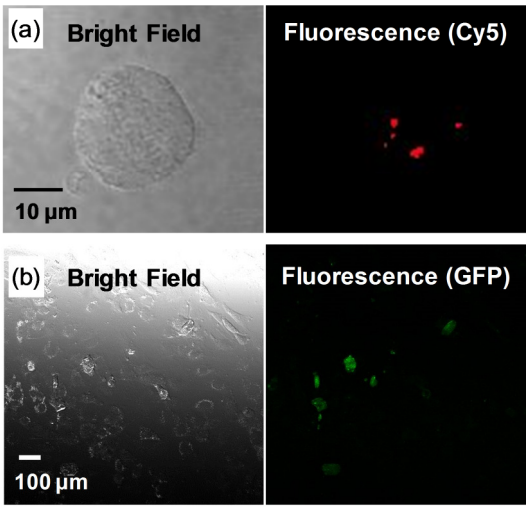

Fig. 1: Bright field and fluorescence images of (a) siRNA-Cy5 transferred MCF-7 and (b) plasmid DNA transferred 3T3L1.

The transfection efficiency of the plasmid DNA is now relatively low, because the large size of the gene is difficult to penetrate the cell membrane. Therefore, we now improve the transfection efficiency by controlling the physical effects such as the electric field associated with charged particles in the diffused plasma and chemical effects associated with plasma-activated products in solution, which are expected to synergistically enhance the endocytosis of the large size of the genes.

[1] S. Sasaki, M. Kanzaki, and T. Kaneko: Appl. Phys. Exp. **7** (2014) 026202.