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Mechanism and Applications of Plasma Gene/Molecular Transfection

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1 Introduction: Molecular/gene introduction by plasma

The authors examined various plasma sources for gene/molecular introduction into cells1. We found that the micro-discharge plasma with a counter electrode provides electrical current to cells in this configuration, simultaneously achieving high transfection efficiency and cell viability[1,2]. Since the plasma treatment time is short as 10^{-2} s or less, damages to cells and plasmid DNA are suppressed.

2 Mechanism: Spontaneous introductions by complex stimuli

The Zeta potential measurement shows that after plasma treatment, cells are charged up by positive charges. Since plasmid DNA, which is naturally charged in negative, collides easily with the charged cell membrane due to the relaxation of the Coulomb repulsion, the collision frequency between plasmid DNA and cell increases. We also experimentally found that large-size molecules such as plasmid DNA are transferred into cells by endocytosis and that ROS and electrical stimuli are required to trigger endocytosis. In our plasma methods, large molecules are transferred into cells by endocytosis, spontaneous cell membrane transfer, by complex stimuli of electric current and ROS[3,4].

In genome editing, it is preferable that the editing molecules are introduced into cells without any random genome integration. The plasma method is expected to be free from random genome integration because cells spontaneously take external DNA molecules up into themselves by plasma-induced endocytosis. The authors proved that the plasma method is random genome integration-free through the experiment[5]. The GFP gene-coded plasmid DNA was introduced into target cells using plasma, electro-poration, or lipofection. The cells were continuously passaged every 3 or 4 days as they reached confluence. After 25 days, many colonies were formed by electro-poration and lipofection methods. On the other hand, only a few colonies were formed by the plasma method. These results prove that plasma treatment introduces a plasmid DNA without random genome integration, so-called "Genome Integration-free."

4 Applications

The plasma induces the spontaneous uptake of cells by endocytosis without random genome integration. The Genome Integration-Free characteristics are unique and expected to be a valuable tool for genome editing. This high level of safety is a feature unique to the plasma method and not found in conventional gene transfer methods. Therefore, the plasma method is expected to enable gene medicine, cell medicine, and regenerative medicine, which could not be realized with conventional gene transfer methods due to a lack of safety. In addition, as clean genome editing is possible, it is also expected to be applied to breeding in agriculture and fisheries.

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