Gene transfer efficiency of surface discharge method depending on molecular size and collision frequency between gene and cell

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Abstract: Through this study, it was suggested that endocytosis is the dominant process of the transfection in surface discharge gene transfection method. We also investigated the relationship between the size of plasmid DNA and the transfection efficiency. According to the numerical calculation it is made clear that when target molecules with a molecular weight of up to 10000 bp, the transfection efficiency is proportional to the collision frequency between target molecule and cell.
Keywords: surface discharge, gene transfection, transfection efficiency, endocytosis

1. Introduction

In molecular biology gene transfection technique is one of the important technologies. It enables many genetic applications such as gene therapy, regenerative medicine, drug development and plant breeding. Currently, various kinds of gene transfection methods are in practical use, however, these conventional methods have some disadvantages such as reagents cytotoxicity and expensive reagents. A safe and highly efficient new gene transfection method which are free from these problems is required. As a method to solve this problem, we have been developing surface discharge gene transfection method. This method has same advantages of low-invasiveness and high-transfection efficiency as the micro-plasma method has and in addition to them, it treat wide area with single operation due to surface discharge.

In this study, we evaluated the contribution degree of endocytosis in the mechanism of gene transfection in the surface discharge method by using endocytosis inhibiter. We also investigated the relationship between the size of target molecule (plasmid DNA) and the transfection efficiency. Based on the result of this experiment, the relation between the transfectioin efficiency and the collision frequency between target molecule and cell are discussed.

2. Method

The schematic of the surface discharge device and electrode arrangement are shown in Fig. 1. The plasmid DNAs introduced into the cells are shown in Table 1. The treatment protocol is as follows: As the target cell HDF (Human dermal fibroblasts) cells were cultured in a 3.5 cm dish. After sucking the medium 120 µl of a plasmid DNA solution (1 µg/µl) was added onto the cells. Half wave rectified sinusoidal wave of 20 kHz is applied to the electrodes and cells are treated by surface discharge. The voltage of 4kV was applied for 5 ms. After surface discharge treatment the medium was added in the dish and the cells in the dish was incubated for 24hrs, then fluorescence observation was carried out by Cytell (GE Healthcare BioScience). When the endocytosis was inhabited, methyl-β-cyclodextrin (MβCD) was added into the medium and incubated for 30 minutes before surface discharge treatment.

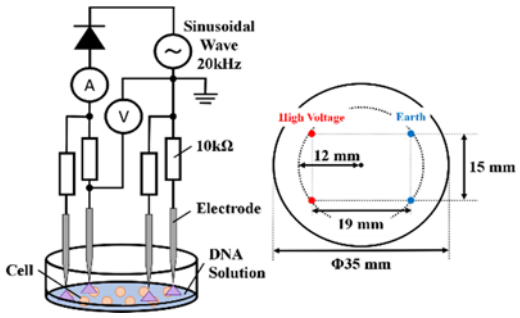

Fig. 1. Schematic of the surface discharge gene/molecular taransfection device and electrodes arrangement.

Table 1. Size of Plasmid DNA

Plasmid DNA	Base pairs / bp	Molecular Weight / MDa
pAcGFP1-N1 (I)	4726	2.95
pCX-EGFP (II)	5510	3.44
pCXLE-EGFP-SmaI (III)	6999	4.37
pCXLE-EGFP (IV)	10912	6.81
pCX-EGFP-tBE (V)	13187	8.23
pCXLE-FLAG-hTET1-PGK-turboRFP-SmaI (VI)	14236	8.88
pCXLE-FLAG-hTET1-PGK-turboRFP (VII)	18149	11.3

3. Results

Figure 2 shows the normalized cell viability and transfection efficiency versus the concentration of the endocytosis inhibitor MβCD. The transfection efficiency at 10 mM of MβCD was 80% lower than that of the case without inhibitor. This means that endocytosis is dominant process in the transfer mechanism of the surface discharge gene transfection method.

The relationship between the number of base pairs (bp) of the plasmid DNA and transfer efficiency is shown in Fig. 3. In this figure the transfection efficiency is normalized by the value of the transfection efficiency of pAcGFP1-N1(8.52%). This figure shows that the transfection

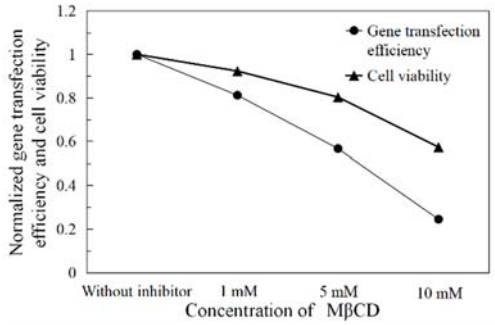

Fig. 2. Normalized gene transfection efficiency of plasmid DNA 2.95 MDa with MßCD

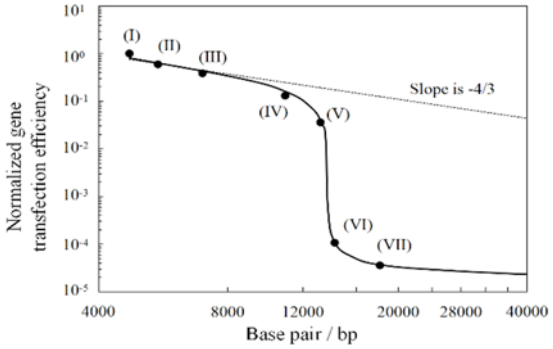

Fig. 3. Relation between transfection efficiency and DNA size and calculated value

efficiency decreased with increasing size of plasmid DNA to be introduced. The decrease in the gene transfer efficiency may be attributed to the following three reasons: decrease in mobility of DNA molecule, decrease in uptake into endocytic pit due to increase in molecular size, and decrease in the number of molecules according to increase in DNA size under same weight concentration. The transfection efficiency for the plasmid DNA over the size of 14 kbp dropped to nearly zero. This suggests that there is an upper limit on the size of DNA that cells can take in by surface discharge method.

4. Discussion

The experimental result of transfection efficiency is quantitatively explained using the following equation [1].

$$D = \frac{RT}{N}\frac{1}{6\pi\eta r} \qquad (1)$$

$$Z = \pi\,(r_{\mathrm{A}} + r_{\mathrm{B}})^2 u_{\mathrm{A}} n_{\mathrm{A}} n_{\mathrm{B}} \qquad (2)$$

As shown in the equation (1), the diffusion coefficient D is obtained from Stokes-Einstein equation. Here R is molar gas constant, T is absolute temperature, N is Avogadro constant, η is viscosity of solution and r is radius of particle moving in liquid respectively. Equation (2) shows the collisions frequency Z between two spheres with different radius and density. Here r_{A} and r_{B} are radius of each sphere, n_{A} and n_{B} are number of particles per unit volume, u_{A} is the velocity of the sphere A. The sphere A representing the target molecule and the sphere B corresponds to the cell. Therefore sphere B has no speed. Based on these equations, the collision between target molecules and cells are discussed. If there are two plasmid DNAs of different molecular weights, the ratio of their molecular weights is denoted as K. The ratio of the number of collisions with cells is given by the following equation.

$$Z_1 : Z_2 = (r + r_1)^2 u_1 n n_1 : (r + r_2)^2 u_2 n n_2 = 1 : K^{-\frac{4}{3}} \qquad (3)$$

Here Z_1 is number of collisions between molecules with small molecular weight and cells, Z_2 is for large molecules, and r and n correspond to cells. Therefore r_1, n_1, r_2 and n_2 correspond to each target molecules. Equation (3) shows that the number of collisions with the cell decreases as the target molecule size increases.

In Fig. 3 the dashed line shows the relation in Eq. (3) and it has a slope of -4/3. The points (I)-(IV) seem to follow this relation. This means transfection depends on the collision frequency between target molecule and cell.

5. Conclusion

It was revealed that endocytosis is the dominant process in the surface discharge gene transfection method. Through the experiment it was suggested that the decrease in the transfection efficiency with increase in the size of DNA is attributed to the decrease of the collision frequency of DNA and cells.

6. Acknowledgement

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7. References

[1] Einstein, A: Annalen der Physik **322** (8), 549–560 (1905).