Investigation for the Mechanism of Plasma Gene Transfection by the Equivalent Circuit Network Analysis Considering Cell Density

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Abstract: In this study, the plasma gene transfection system including the cells, DNA solution and a 96-well plate was modeled by an equivalent circuit network. Analysis showed that the maximum transfection efficiency was obtained at the same intracellular current density, suggesting that the intracellular current density is a key factor inducing the gene transfer. In addition, at the same intracellular current density, higher cell density is preferred probably because of lower cell damages by reactive species.

Keywords: gene transfection, transfection efficiency, endocytosis

1. Introduction

The authors have developed a plasma gene delivery method using micro-discharge plasma as a highly efficient, minimally invasive, and safe gene delivery method [1]. In this technique, the authors have performed an equivalent network analysis to clarify the effect of the intracellular current density on the transfection efficiency of the plasma gene transfer method. There are well-known examples of electrical circuit analysis modeling a single cell, in which cells are modeled with the cell membrane as a capacitor and the cytoplasm as a resistance [2]. In a previous study, we have developed an equivalent RC circuit network model that models a whole gene transfection system composed of many cells, plasmid solution and a 96-well plate. The analysis showed that current is probably the primary source of electrical stimulation to cells [3]. In this study, the model was improved to include plasmid solution between cells to model the cells cultured with low density, and the model's validity was verified.

2. Method

Figure 1 shows a schematic diagram of the experimental apparatus. Mouse-derived fibroblasts (L-929) were cultured on 96-well plates and the medium was replaced with GFP plasmid solution (4μg / 4μl) before plasma treatment. The 96-well plate was placed on a grounded electrode plate and was exposed to micro-discharge plasma for 20 ms; the plasma was generated by applying a 20 kVpp, 20 kHz sinusoidal voltage to an ultrafine electrode positioned 0.5 mm above the liquid surface at the center of a well. The green fluorescence expressed by plasmid transfection was observed after 24 hours of incubation, and the transfection rate was calculated.

The phenomenon in the well was assumed to be axisymmetric, and an equivalent circuit was designed by dividing the well into 16 radial sections (Δr = 0.2 mm in one unit). Each section consisted of two layers: the upper (plasmid) and the lower (cell) layers. Figure 2 shows one unit of the lower (cell) layer in the 16 divisions. The cell density was expressed as the ratio of the cell area (yellow-highlighted area in Fig. 2). The plasma was assumed to be a perfect conductor, and a sinusoidal voltage source was connected to the buffer solution at the center of the well. The radial distribution of the intracellular current under the steady state condition was calculated with LTspice.

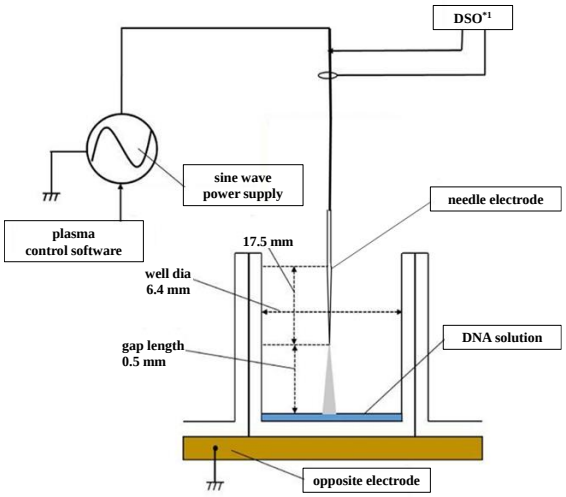

Fig. 1. Schematics diagram of plasma treatment

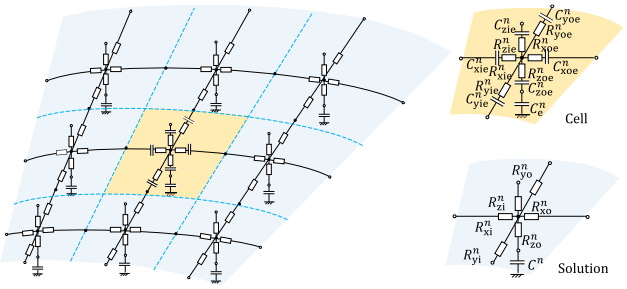

Fig. 2. Equivalent circuit (lower layer of 1 unit)

3. Results

The calculated intracellular current density values for each cell density are shown in Figure 3. The relationship between measured values of transfection efficiency / survival rate and calculated intracellular current density are

shown in Figures 4 and 5. The smaller the cell density is, the higher the intracellular current density is obtained. As shown in yellow highlight in Figure 4, regardless of the cell density, the optimum intracellular current density, at which the maximum transduction rate is obtained, is almost constant around 9 A·m^{-2}. At the same intracellular current density, higher transfection efficiency and higher survival rate are obtained for the higher cell density.

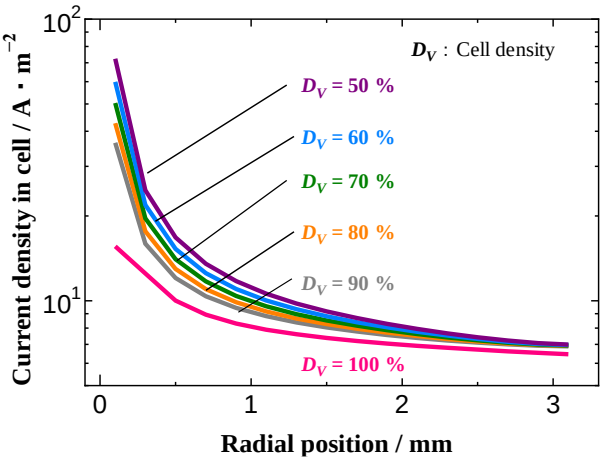

Fig. 3. Radial distribution of intracellular current density at various cell densities

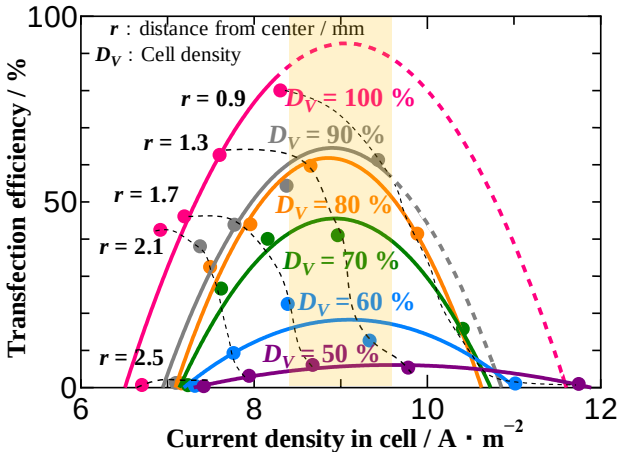

Fig. 4. Dependence of introduction efficiency on intracellular current density.

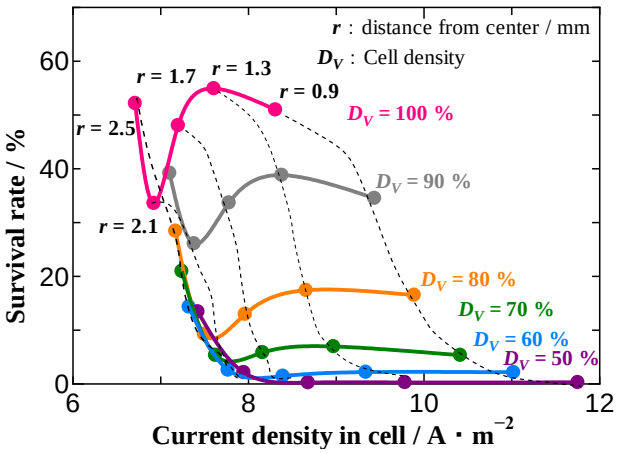

Fig. 5. Dependence of survival rate on intracellular current density.

4. Discussion

Under all the conditions of cell density, maximum transfection efficiencies are obtained at the same intracellular current density. This result suggests that the intracellular current density is the key factor to induce the gene transfection. However, the maximum transfection efficiency value differs according to the cell density: higher transfection efficiency is obtained at the higher cell density. We assume that it is attributed to the reactive species generated in the buffer solution. At the same current density, the same number of reactive species are generated. That means larger number of reactive species affect a cell if the number of cells is small. Thus, the excess stimuli by the reactive species damage the cells, resulting in lower transfection efficiency under the low cell density condition.

5. Conclusion

An equivalent circuit network model of the plasma gene transfection system considering cell density has been developed. The simulation results suggest that intracellular current density is a key factor inducing the gene transfection. At the same intracellular current density, higher cell density is preferred probably because of lower cell damages by reactive species.

6. Acknowledgement

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7. References

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