Positive and negative effects of ROS in micro-discharge plasma gene transfer method

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Abstract: In this study, ROS inhibition after gene transfer with micro-discharge plasma treatment was performed. By the ROS inhibition, a decrease in the number of dead cells and an increase in the number of expressing cells were observed. These results suggest that the ROS inhibition after gene transfer in the plasma method improves the performance by reducing the cytotoxicity of the ROS.

Keywords: gene transfer, ROS, endocytosis

1. Introduction

We have been developing plasma gene transfer, a new gene transfer method using gas discharge [1]. The advantage of this method is that the plasma does not need to cause membrane perforation and only triggers endocytosis. In addition, since the energy required to trigger endocytosis is small, the plasma is minimally invasive to the cells. We have already reported that electrical and chemical stimuli combined induce endocytosis. Our previous studies have also shown that as the chemical stimuli, reactive oxygen species (ROS) are required [2]. However, it is generally accepted that ROS has harmful effects on cells [3]. In this study, we will clarify the effect of the cytotoxicity of ROS generated by plasma in the plasma gene transfer method.

2. Method

Figure 1 shows a schematic diagram of the experimental setup. L-929 cells, which are mouse fibroblasts, were used as target cells. The plasmid solution was dropped into cultured cells and treated with the discharge plasma. N-acetyl-L-Cysteine (NAC) and Catalase were used as ROS inhibitors, and each inhibitor was diluted in serum medium and added to cells after plasma treatment. We also performed a reagent-free ROS removal experiment using a condition in which the cells were washed with a culture medium after the discharge treatment (culture medium wash condition). In all conditions, the cells were stained with Hoechst 33342 and propidium iodide after 24 hours and observed for fluorescence.

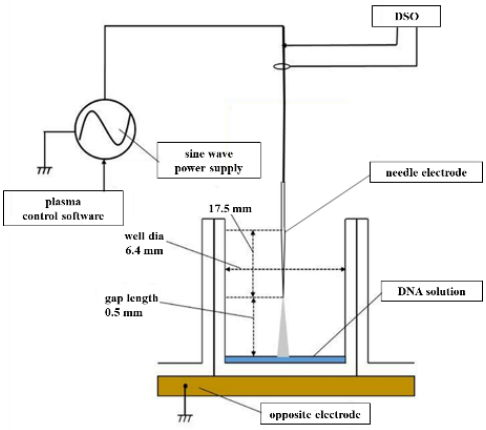

Fig. 1. Schematics diagram of plasma treatment

3. Results

The fluorescence images taken with a fluorescence microscope are shown in Fig.2. The green fluorescence shows the expression of the pacgfp1-N1 plasmid. The red fluorescence shows the dead cells stained with propidium iodide. The numbers of nuclear stained cells, expressed cells, and dead cells are shown in Fig.3. Under NAC conditions, the number of expressed cells increased by 43%, and the number of dead cells decreased by 61% compared to the control condition. Under catalase conditions, the number of expressed cells increased by 61% compared to the control condition. Under the medium wash condition, the number of dead cells was reduced by 56% compared to the control condition.

4. Discussion

The reductions in the number of dead cells under NAC and medium wash conditions were observed. These results suggest the reduction of ROS after plasma treatment reduces cytotoxicity of the ROS. On the other hand, in the catalase condition, the reduction of the number of the dead cell was not observed. This result suggests that ROS other than hydrogen peroxide, inhibited by catalase, is the main player in cytotoxicity. Another possible explanation is that the inhibition effect was insufficient at this inhibitor concentration. Therefore, we plan to measure ROS inside and outside the cell in the future.

The increases in the number of expressed cells observed with both NAC and catalase suggest that hydrogen peroxide may inhibit expression.

5. Conclusion

Plasma-generated ROS were found to be involved in transduction and cytotoxicity. These results suggest that by controlling the amount of ROS, further high efficiency and expression of plasma gene transfer are expected.

6. Acknowledgement

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7. References

[1] M. Jinno et al., Jpn. J. Appl. Phys., **55**, 07LG09 (2016)

[2] M. Jinno et al., Jpn. J. Appl. Phys., **60**, 030502 (2021)

[3] S.G. Rhee, Science, **312**(5782), 1882 (2006)

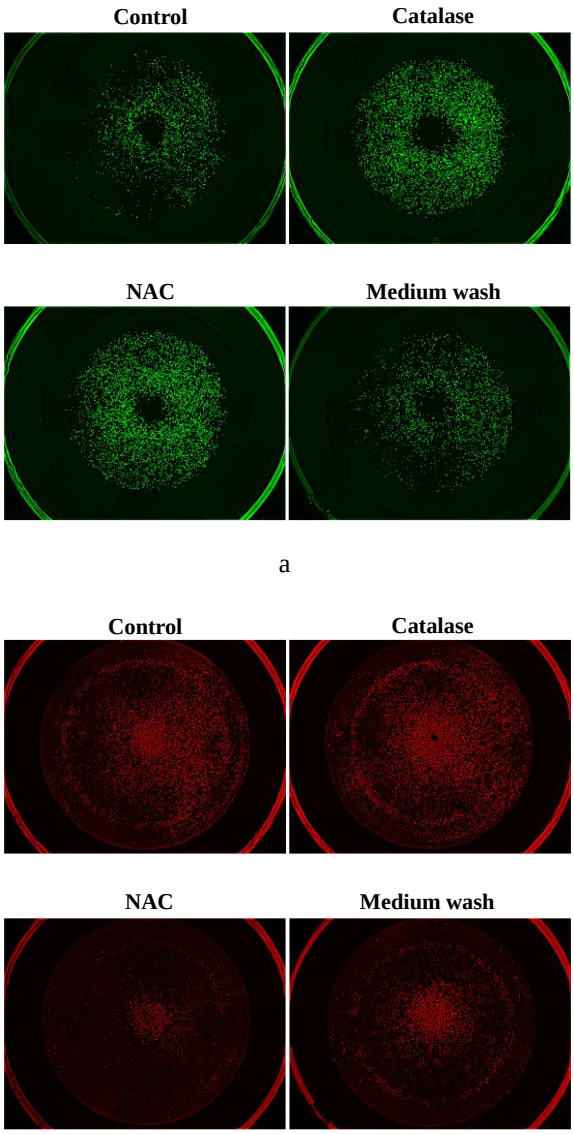

Fig. 2. Fluorescence images: (a) Expressed cells fluorescence image. (b) Dead cells fluorescence image

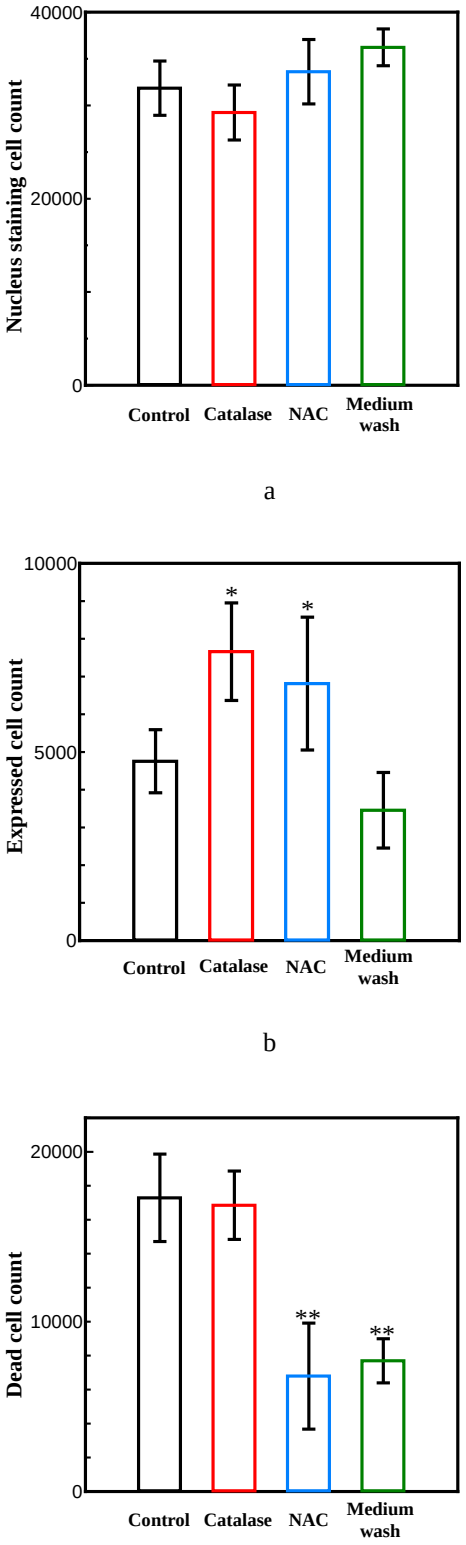

Fig. 3. Measurement results (*: $p < 0.05$, **: $p < 0.01$): (a) Nucleus staining cell count. (b) Expressed cell count. (c) Dead cell count.