Plasma molecular introduction into plant cells: Differences with animal cells

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Abstract: Molecular introduction into plant callus via plasma treatment requires both plasma-generated electrical stimuli, such as current, and chemical stimuli, like ROS. Molecules are introduced through plasma-induced spontaneous uptake, i.e. endocytosis. Physical entry pathways in the cell wall are necessary for spontaneous uptake. The introduction efficiency depends on the frequency and duration of plasma treatments.

1. Introduction

Plant cells are surrounded by a cell wall, making direct introduction of molecules such as proteins more difficult than in animal cells. However, the authors have successfully introduced a Cas9 and sgRNA complex into tobacco callus (Nicotiana tabacum) using a micro-plasma method for genome editing [1] and have also successfully introduced and expressed a GUS plasmid. In this presentation, we report on the role of plasma in introducing molecules into plant cells.

2. Methods

Tobacco callus cells were used as targets. The fluorescent molecule FITC-dextran (Sigma-Aldrich: 250 kDa) was used as the transfection material. The tobacco callus was placed in a 3.5 cm dish placed on a ground electrode, and an HV electrode was placed 1 mm above the callus. 11 kVpp sinusoidal voltage was applied for plasma treatment, 5 µl of FITC-dextran solution was dropped onto the irradiated area, allowed to stand for a specific time, washed and observed using a fluorescence microscope. The cells were washed and observed under a fluorescence microscope.

3. Results and Discussion

Electron microscopy images show that plasma forms gaps on the surface of the cell wall in the case of successful molecular introduction. The molecules must pass through this gap to reach the cell membrane. In N-Acetyl-L-cysteine (NAC)-treated tobacco callus, both plasma-generated extracellular ROS and plasma stress-induced intracellular ROS are inhibited. In this case, no molecules are introduced, even if the plasma treatment forms gaps in the cell wall. This means that even if the molecules reach the cell membrane, endocytosis is not induced due to the inhibition of ROS, and they are not introduced into the cell.

Molecules are not introduced when tobacco callus is treated with helium plasma jets. While the helium plasma jet produces ROS and provides chemical stimulation to tobacco callus, the current stimulus provides little to no current stimulus to the cells because there are no target cells on the current pathway from the power source. This means that chemical stimulus by ROS alone does not induce endocytosis, and, as in animal cells, both current and chemical stimuli are required.

Multiple plasma treatments resulted in fluorescence maxima for certain treatment times and times. For the introduction of molecules into plant cells, it is important that the cells are subjected to multiple plasma treatments. This creates gaps in the cell wall surface and provides the necessary stimuli for the induction of endocytosis. Thus, plasma molecular introduction into plant cells requires multiple treatments, a plasma treatment different from that for animal cells.

4. Conclusion

This study shows that the introduction of molecules into plant cells by micro-plasma treatment requires the formation of gaps in the cell wall that function as pathways for molecules to reach the cell membrane and that the induction of endocytosis in plant cells requires both electric current stimulus by plasma and chemical stimulus by active species, as in animal cells.

Acknowledgement

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References

[1] Y. Ikeda et al., *Jpn. J. Appl. Phys*., **62**, SL1015 (2023).

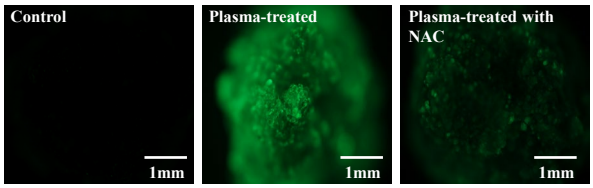

Fig. 1. Fluorescence image of tobacco callus: plasma-treated callus emits green fluorescence, whereas NAC-treated callus emits little fluorescence.

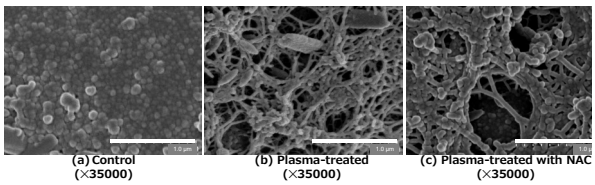

Fig. 2. SEM image of tobacco callus surface: Gaps are formed on the cell wall surface by plasma treatment.