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Temperature-Lowered Plasma Treatment by Controlling Direction of Supplying Reactive Species for Biological Application

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Abstract

Temperature-lowered plasma treatment was performed against biological samples by using a MEMS nozzle device and optimizing the direction of supplying reactive species in the plasma. During the plasma treatment, the temperature was 40°C, which was almost the same as human body. The plasma treatment was applied to pollen of *Lathyrus odoratus*. The plasma treatment induced no significant changes on the surface of pollen but the ratio of germination was decreased. The plasma treatment seemed to act on the inside of the pollen and affect the biological functions.

1. Introduction

Low-temperature atmospheric pressure plasma has been applied to biological treatment [1-4]. The plasma treatment constitutes a fast growing area in plasma research. The plasma treatment should use interaction between the plasma and cells. Basically, the low-temperature plasma was irradiated onto a broad area of a tissue. Cells included in the area were affected. To induce the biological functions of a cell individually, the area of plasma irradiation should be reduced to the level of the size of a cell (20–100 μm).

So far, we have achieved localized plasma treatment of individual cell by using a MEMS nozzle device [5]. The surface of biological samples was locally etched by plasma treatment (ϕ5–30 μm). For the biological and/or medical applications, temperature of plasma treatment should be low not to thermally damage the biological sample. For example, skin of mice was thermally damaged when heated at 44 ºC [6]. In the present study, temperature of plasma treatment using a MEMS nozzle device was lowered by optimizing the direction of supplying reactive species in the plasma. The temperature-lowered plasma treatment was applied to biological samples.

2. Principle

Localized plasma treatment was performed by attaching a MEMS nozzle device to an output port of an atmospheric pressure inductively coupled plasma source as shown in Fig. 1. By operating a pump, a biological sample is trapped at the MEMS nozzle. The MEMS nozzle device has through-holes at the bottom membrane. The through-holes define the area of plasma irradiation. In this configuration, plasma is generated in the upstream region from the biological sample. The reactive species are supplied to the sample along the regular flow of He gas. This configuration is named regular flow type and suitable to effectively supply the reactive species. However, the reactive species in the plasma continuously impinge onto the biological sample. The sample should be heated up easily.

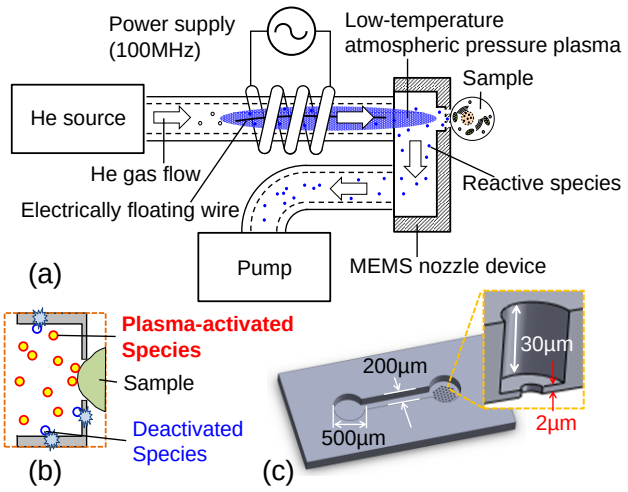

Fig. 1. (a) Schematic drawing of localized plasma irradiation to a biological sample. (b) Magnified image of plasma irradiation at the nozzle device. (c) Schematic illustration of MEMS nozzle. At the bottom, through-holes are prepared.

We modified the position of plasma generation to the downstream region as shown in Fig. 2. An electrically floating wire is set inside a tube of downstream region. When VHF power is supplied to the coil antenna, the floating wire is excited to high potential by the inductive coupling, enhancing the plasma ignition [7]. The plasma is generated in the downstream side. The reactive species in the plasma diffuse towards the biological sample, opposing to the regular flow of He gas. This configuration is named counter flow type. Because He gas at room temperature is continuously supplied to the MEMS nozzle device and the

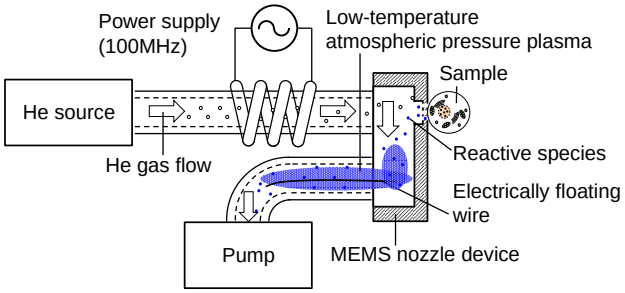

Fig. 2. Experimental configuration of counter flow type.

sample, the MEMS nozzle device and the sample are always cooled down to room temperature.

3. Experiments

Fabrication sequence of the MEMS nozzle device was described in detail elsewhere [7]. The MEMS nozzle device was attached to the output port of the plasma source and also to the port of gas evacuation with glue. Temperature of MEMS nozzle was measured by thermography. To identify the reactive species in the plasma, optical emission spectroscopy was performed.

For the biological experiments, pollen of *Lathyrus odoratus*, which had short germination time, was used. An agar gel was prepared and cut into pieces of 1cm^3. The pollen of *Lathyrus odoratus* was put on the agar piece and plasma treatment was conducted. The plasma-treated pollen was incubated for one day and observed by optical microscopy.

4. Results and discussion

With regard to the regular flow type, plasma irradiation temperature was 100°C at the MEMS nozzle part as shown in Fig. 3(a). Comparing to the damaging temperature of skin of mice (44ºC) [6], 100ºC was much higher. Careful treatment should be needed for the biological application. In contrast, the temperature of counter flow type was about 40ºC at the MEMS nozzle device as shown in Fig. 3(b). More than two-fold reduction was indeed achieved. Optical spectroscopy revealed O and OH radicals in the plasma (data not shown). Those radicals can affect the biological function of target cell.

Using the counter flow type, pollen of *Lathyrus odoratus* was plasma-treated for 20 min. However, as shown in Figs. 4(a) and 4(b), the traces of plasma treatment were not found by optical spectroscopy. Thus, we incubated the pollen and analyzed the ratio of germination. As shown in Figs. 4(c) and 4(d), the pollen treated by plasma showed low germination. The germination ratio of the plasma-treated sample was evaluated to 3.3%. On the other hand, the germination ratio of the control sample, which was not plasma-treated, was 21.8%. The plasma treatment seemed to act on the inside of the pollen and affect the biological functions.

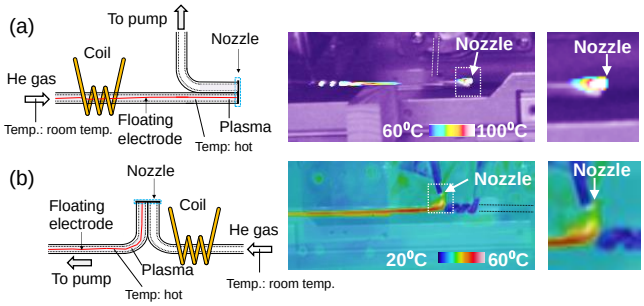

Fig. 3. Experimental setups and thermal images of (a) regular flow type and (b) counter flow type configurations.

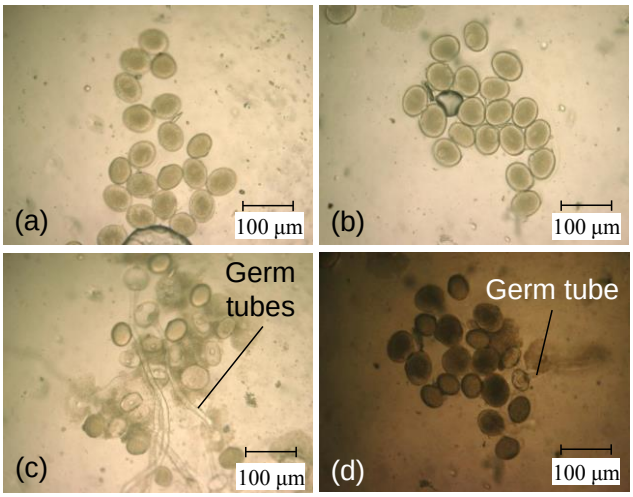

Fig. 4. Optical microscopy images of the pollen of *Lathyrus odoratus* of (a, c) control and (b, d) plasma-treated samples. After one day incubation, germ tubes grew from the pollen (c,d).

5. Conclusions

Localized low-temperature plasma treatment was achieved by using a MEMS nozzle device and optimizing the methods of supplying reactive species in the plasma and cooling the MEMS nozzle. Temperature of the plasma treatment was decreased to 40ºC. Plasma treatment using counter flow type showed no significant changes on the surface of pollen of *Lathyrus odoratus*, but decreased the ratio of germination.

Acknowledgements

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