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Controlling feeding gas temperature of plasma jet with Peltier device for experiments with fission yeast

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The suitable temperature condition for fission yeast growth is around 30 °C, whereas the gas temperature of commonly used atmospheric-pressure helium plasma jets tends to increase with discharge time. To avoid the damage of fission yeast from heat, a Peltier device was used to control the feeding gas temperature of low-frequency helium plasma jet. It was attained that the temperature of a thermistor directly irradiated by the plasma jet plume was almost constant at the acceptable temperature to fission yeast for more than 200 s. The morphology of fission yeast cells exposed to the gas-temperature-controlled plasma jet plume was examined. Some of the cells in the proximal of plasma irradiation point showed elongated shape, which is not seen in wild-type fission yeast cells under normal conditions.

1. Introduction

Recently, non-equilibrium atmospheric pressure plasmas have widely been studied in connection with a variety of applications such as plasma medicine¹⁻³⁾ and plasma agriculture⁴⁾. Well-known examples of positive effects are apoptosis induction to cancer cells by plasma-activated medium (PAM)⁵⁻⁹⁾ and germination and growth promotion of plants by direct plasma irradiation to seeds.¹⁰⁻¹²⁾ Further development of plasma-bio applications requires deeper understanding of fundamental mechanisms based on cellular and molecular biology. In order to apply biological methodology to plasma-bio research, some of the model organisms, e.g. thale cress (*Arabidopsis thaliana*)¹²⁾ and budding yeast (*Saccharomyces cerevisiae*)¹³⁻¹⁶⁾, have been used in preceding studies.

In the light of recent research demands, we chose fission yeast (*Schizosaccharomyces pombe*)¹⁷⁻²⁰⁾ as a model organism to evaluate the effects of direct plasma treatment on eukaryotic cells. Fission yeast is a rod-shaped unicellular eukaryote, and it grows by tip extension and divides by medial fission. Its highly reproducible cell size and defined shape enable us to study the cell cycle. Acceptable temperature for growth of fission yeast is from 20 to 35 °C.²¹⁾ Moreover, careful temperature control is indispensable, especially when using temperature-sensitive mutants, whereas the neutral gas temperature of commonly used atmospheric-pressure plasma jet tends to increase in the course of discharge.

The need to control the gas temperature of atmospheric-pressure plasma has always been recognized, and several temperature-controllable plasma devices have been developed in which the feeding gas was cooled by liquid nitrogen.²²⁻²⁴⁾ Here, we adopted a Peltier device to control the feeding gas temperature. The Peltier device can convert electric voltage to temperature difference by thermoelectric effect. Therefore, it can work as a cooler or as a heater by only changing electric voltage. Gas temperature control with no need for liquid nitrogen makes the plasma device an easy-to-use tool for plasma-bio experiments.

In this paper, we report the development of low-frequency (LF) helium plasma jet with a Peltier device and initial results of direct plasma irradiation to fission yeast, *S. pombe*. Portions of this work were presented at the 40th International Symposium on Dry Process in 2018 and published as a proceedings paper.²⁵⁾ Microscopic observations of fission yeast exposed to gas-temperature-controlled plasma jet plume have been included as new materials.

2. Experimental methods

2.1 Atmospheric-pressure helium plasma jet system with a Peltier device

Figure 1 shows the schematic of the LF plasma jet with a Peltier device that actively cools and heats the feeding gas. The helium gas was supplied through a copper pipe (4 mm in outer diameter and 2 mm in inner diameter) attached to the Peltier device (VICS, LVPU-30) in which the maximum endothermic quantity was 18 W. The copper pipe was connected to a dielectric (glass) tube of 55 mm long with the same diameters. An electrode (15 mm-wide copper tape) was put on the glass tube at a distance 15 mm from the nozzle. The electrode was connected to the high voltage power supply (LOGY ELECTRIC, LHV-10AC-24). This plasma source has no electrically grounded electrode, i.e. a single electrode LF plasma jet²⁶⁻²⁸. Because of the high heat conductivity of helium, the whole unit was thermally insulated by polystyrene foam. A typical waveforms of the applied voltage and discharge current are shown in Fig. 2, where the voltage and the current were measured with a high voltage probe (Tektronix, P6015A) and a standard CT for wide frequency bandwidth (URD, CTL-35-S162-5F-1R10), respectively. The input power (P_{in}) was estimated as follows: (i) integrating the product of voltage (broken line in Fig. 2) and current (solid line in Fig. 2) for a period (66 μ s), and (ii) multiplying the result by the repetition frequency (15.1 kHz). In the case of Fig. 2 (the peak-to-peak voltage $V_{p-p} = 11.0$ kV), the calculated input power was $P_{in} = 4.0$ W. Although the voltage waveform is not a sinusoidal one, the input power is almost linearly proportional to V_{p-p} . The voltage from the ground to positive peak (V_{g-p}) can also be used as a reference of input power. The minimum input power that can sustain the plasma jet was $P_{in} = 0.6$ W, where $V_{p-p} = 5.4$ kV and $V_{g-p} = 3.4$ kV. The helium gas flow rate was adjusted by a mass-flow controller at 3 standard liters per minute (slm). In this work, the temperature of a NTC thermistor (THORLABS, TSP01) directly irradiated by the plasma jet plume was used as an index of the gas temperature in the plasma plume and called as “the gas temperature”.

2.2 Fission yeast strain, media and cell culture

The fission yeast *S. pombe* strain JY450 (genotype: h^{90} *ade6-M216 leu1*), which is a wild-type strain derived from a single original standard strain¹⁹, was used. The wild-type JY450 cells were re-isolated from our lab stock at each experiment, thereby ensuring that all cells in this study are genetically identical. 10 ml of vegetative cells ($4 - 6 \times 10^6$ cells/ml) in complete media (YEA: yeast extract with adenine) were harvested by filtration²⁹ (Millipore DURAPORE Membrane filters, DVPP04700, 0.65 μ m). The pellet of fission yeast on the membrane filters were exposed to plasma jet plume or helium gas at room temperature (25 $^{\circ}$ C $\pm$ 1 $^{\circ}$ C). The filters were then placed onto YEA plates and incubated at 30 $^{\circ}$ C. The cells

after 1-5 day incubation were observed under a microscope (Axioplan 2, Carl Zeiss, Oberkochen, Germany). Images were captured using a chilled CCD camera (CoolSNAP HQ2, Photometrics, Tucson, AZ, USA) and MetaMorph software (Molecular Devices, Sunnyvale, CA, USA).

3. Results and discussion

3.1 Gas temperature control of helium plasma jet

At first, the gas temperature in plasma plume without gas cooling was investigated. The thermistor was placed on a plate at a distance of 20 mm from the nozzle and directly irradiated by the plasma jet plume of which length was more than 30 mm. Figure 3 shows the time evolution of the gas temperature by discretely changing the input powers. It is clearly seen that the gas temperature sharply increases to more than 70 °C ($V_{p-p} = 11$ kV and $P_{in} = 4.0$ W) and reaches a certain equilibrium temperature. Subsequently, the gas temperature decreases corresponding to the decrease in input power. However, the equilibrium temperature at the minimum input power that can sustain plasma jet is 37 °C in this experiment, which is slightly higher than the acceptable temperature of fission yeast, i.e. 20-35 °C.

In order to adjust the gas temperature of plasma jet, the feeding gas was cooled by the Peltier device in advance. Figure 4 shows the relation between the input power and the gas temperature with different initial gas temperatures. The initial gas temperatures with and without gas cooling were 16.2 °C (The setting temperature of the Peltier device: 2 °C) and 27.9 °C, respectively. In both cases, the gas temperature is linearly dependent on input powers with the same proportionality constant. This result ensures that the gas temperature of the plasma jet can be satisfactory controlled by the initial gas temperature within the cooling capability of the Peltier device. Figure 5 demonstrates the controllability of gas temperature by the Peltier device. When the input power was 0.6 W and the setting temperature of the Peltier device 2 °C, the equilibrium temperature was 23 °C, which was slightly lower than the room temperature 25 °C. By changing the setting temperature from 2 °C to 5 °C, the gas temperature increased to 25.2 °C and remained steady for 240 s with the standard deviation of ± 0.2 °C. This is a suitable temperature condition for treating fission yeast without damage from heat. It should be mentioned that the setting temperature of the Peltier device should be changed to the room temperature after terminating the discharge, because the gas temperature of 16.2 °C is not suitable for irradiating to the control cells.

Although we fixed the gas flow rate to 3 slm in our experiment, it can be considered as

another control knob of the gas temperature unless the flow scatters the irradiation target, because high flow rate decreases the input power per unit volume. However, the gas flow rate as well as the input power may affect the plasma property such as electron density and electron temperature, and consequently the radicals produced by this plasma jet. Quantitative evaluation of plasma properties is left for our future work.

3.2 Morphology of fission yeast exposed to gas-temperature-controlled plasma jet

Fission yeast cells on a filter were exposed to plasma jet plume for one minute with the distance of 20 mm from the nozzle. Discharge voltage and gas flow rate were $V_{p-p} = 5.4$ kV and 3.0 slm, respectively. The gas temperature in the plasma plume was adjusted to 25 °C by cooling the feeding gas with the Peltier device. After terminating the plasma discharge, cells on another filter were exposed to helium gas flow of which temperature was adjusted to 25 °C as a control. Figure 6 (a) shows the image of the plasma-treated filter after 5 days of incubation at 30 °C. Fission yeast cannot grow in the plasma plume irradiation point denoted by proximal 1 (dark region of which size is comparable to the diameter of plasma jet plume). Proximal 1 is surrounded by proximal 2 in which subtle growth inhibition can be seen. Far from proximal 1 is denoted by distal which exhibits no obvious growth inhibition. Figure 6 (b) shows the image of a control filter exposed to helium gas flow of which temperature was the same as that of plasma plume (25 °C). Subtle growth inhibition can also be seen in the gas irradiation point denoted by proximal 1.

Figure 7 shows the microscope images of fission yeast cells sampled from the same filters exhibited in Fig. 6 after 5 days of incubation: (a: proximal 1 of Fig. 6 (a)) Most of cells are broken in the area just hit by plasma jet plume. (b: proximal 2 of Fig. 6 (a)) A mixture of dead, healthy and elongated cells is seen. It should be emphasized that such elongated shape is not seen in wild-type cells under normal growth conditions. (c: distal of Fig. 6 (a)) No obvious effect of plasma exposure is observed. (d: proximal 1 of Fig. 6 (b)) Control cells only exposed to helium gas show healthy growth and reach growth saturation as with Fig. 7 (c).

Figure 8 shows an example of elongated cells observed in proximal 2 of Fig. 6 (a) after one day incubation at 30 °C. Even in the proximal 2, normal cells are still active and form a septum (cell plate), which is a previous step of cell division. On the other hand, the elongated cell shows no symptom of cell division, which suggests that the cell cycle control may be impaired by plasma exposure. We are currently investigating this possibility by using cell

division cycle (*cdc*) mutants and will present detailed analyses in a future article. It should be mentioned that it is still an open question that why only a small amount of cells are affected by the plasma treatment. Quantitative analysis of the production of reactive oxygen species (ROS) is indispensable for further study.

4. Conclusions

We have successfully controlled the gas temperature of low frequency plasma jet by cooling the feeding helium gas with a Peltier device. By adjusting setting temperature of the Peltier device while keeping the input power and the gas flow rate constant, the suitable temperature condition for fission yeast (*S. pombe*) was attained for more than 200 s. This enables us to perform experiments on the effects of plasma irradiation to fission yeast without damages from heat.

The microscopic observations of fission yeast cells exposed to gas-temperature-controlled plasma jet plume have revealed that some of the cells in the proximal of plasma irradiation point exhibit elongated shape which is not seen in wild-type cells. It is suggested that the cell cycle control may be impaired by plasma exposure. Detailed mechanisms underlying this morphological change will be studied by further biological analysis.

Acknowledgments

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Figure Captions

Fig. 1. (Color online) Schematic of LF plasma jet with a Peltier device to control the feeding gas temperature.

Fig. 2. (Color online) A typical waveforms of applied voltage (broken line, blue online) and discharge current (solid line, red online). The input power was calculated by integrating the voltage and current for a period and multiplying that by the repetition frequency.

Fig. 3. (Color online) Time evolution of the gas temperature by changing input powers discretely without initial gas cooling. A certain equilibrium temperature exists for each input power.

Fig. 4. (Color online) The dependence of gas temperature on input power with (closed square) and without (closed circle) gas cooling by Peltier device. The linear dependences with the same proportionality constant are evident.

Fig. 5. (Color online) Realization of a suitable gas temperature condition for fission yeast experiment by adjusting the setting temperature of Peltier device. More than 200 s stability was attained.

Fig. 6. (Color online) Images of membrane filters after 5-day incubation at 30 °C. (a): One minute treatment with the room temperature helium plasma jet. (b): One minute treatment with the room temperature helium gas as a control. The irradiation point is denoted as proximal 1. The area in the vicinity of proximal 1 in (a) is denoted as proximal 2. The outer region is denoted as distal.

Fig. 7. (Color online) Microscopic images of fission yeast cells after 5-day incubation at 30 °C. (a): Most of the cells are broken in proximal 1 of plasma-treated filter (Fig. 6 (a)). (b): A mixture of dead, healthy and elongated cells is seen in proximal 2 of plasma-treated filter (Fig. 6 (a)). (c): The cells show healthy growth and reach saturation in distal of plasma-

treated filter (Fig. 6 (a)). (d): The control cells only exposed to helium gas show healthy growth and reach saturation even in the proximal 1 of Fig. 6 (b) as with the distal of plasma-treated filter.

Fig. 8. (Color online) Microscopic image of plasma-treated cells in proximal 2 of Fig. 6 (a) after 1-day incubation at 30 °C. Normal cells form a cell plate which is a previous step to cell division. The elongated cell shows no such symptom, which suggests that the cell cycle may be impaired.

Fig.1. (Color online)

Fig. 2. (Color online)

Fig. 3. (Color online)

Fig. 4. (Color online)

Fig. 5. (Color online)

Fig. 6. (Color online)

Plasma-treated cells (Proximal 1)

Plasma-treated cells (Proximal 2)

Plasma-treated cells (distal)

Helium-treated control cells (Proximal 1)

Fig. 7. (Color online)

Fig. 8. (Color online)