Exposure to helium plasma jet induces cell elongation of fission yeast

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Abstract: A helium plasma jet system with a feeding gas cooling section has been developed to perform biological experiments on direct plasma-jet irradiation effects to fission yeast. The gas temperature in the plasma plume is controlled by a Peltier device to be kept at the room temperature. It is found that one-minute exposure to the plasma jet induces cell elongation to a few percent of fission yeast in the proximal of irradiation point. The phenotype suggests that cell cycle control may be impaired by the plasma exposure.

Keywords: fission yeast, room temperature plasma jet, direct irradiation, cell elongation

1. Introduction

Recently, applications of low-temperature atmospheric-pressure plasmas have been actively studied in medical and agricultural field, and molecular biological understanding on fundamental processes of interaction between plasma and living organisms is demanded. To facilitate the use of established techniques in cell biology and genetics, several model organisms have been utilized in plasma-bio research field, e.g. *Arabidopsis thaliana* in germination and growth promotion by plasma irradiation to seeds [1].

In our study, fission yeast (*Schizosaccharomyses pombe*) [2, 3], which is one of popular model organisms, has been chosen to examine the effects of direct plasma irradiation on eukaryotic cells. Since permissible temperature for growth of fission yeast is from 20 to 35 °C [4], it is required to control the gas temperature of plasma plume during irradiation. We have developed a single-electrode helium plasma jet system with a Peltier device that actively cools the feeding gas [5]. A suitable temperature condition for irradiation to fission yeast can be achieved by adjusting the setting temperature of Peltier device while the input power and the gas flow rate are kept constant.

In this paper, we report a morphological change observed in some fission yeast cells directly exposed to plasma plume.

2. Experimental methods and materials

2.1 Gas-temperature-controlled plasma jet system

The plasma jet has a conventional single-electrode configuration with no electrically grounded electrode [6]. Helium gas is supplied through a 2 mm inner diam copper pipe attached to the cooling section with a Peltier device. The pipe is connected to a glass tube of 55 mm in length with the same inner diameter. A 15 mm wide copper tape wound on the glass tube at a distance 15 mm from the nozzle is used as an electrode. The gas flow rate, applied high voltage, and frequency in the present experiment were 3 slm, 5.4 kV peak-to-peak, and 15 kHz, respectively. The input power was 0.6 W. Under the above conditions, the gas temperature of plasm plume measured by a thermistor located at a distance of 20 mm from the nozzle was room temperature (25 °C).

2.2 Fission yeast

A wild-type fission yeast (*S. pombe*) strain JY450 (h^{90} *ade6-M216 leu1*) was used. 10 ml of vegetative cells (4 - 6 × 10^6 cells/ml) in YEA (yeast extract with adenine) media were harvested by filtration (Millipore DURAPORE Membrane filters, DVPP04700, 0.65 μm).

2.3 Experimental procedure

The cells on a filter were directly exposed to gas-temperature-controlled plasma plume with the distance of 20 mm from the nozzle for one minute. As shown in Fig. 1, the membrane filter was placed on agar plate to prevent

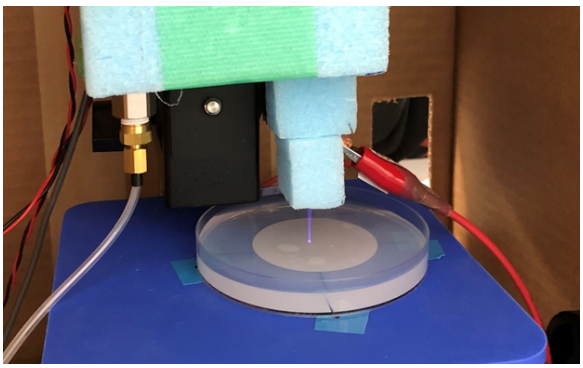

Fig. 1. Fission yeast cells on a filter was directly exposed to plasma plume of which gas temperature was controlled to 25 °C ± 1˚C.

from drying during plasma irradiation period. After plasma treatment, the filters were placed on new YEA plates and incubated at 30 °C. The morphology of the cells after 1-5 day incubation was observed under a microscope.

3. Results and discussion

Figure 2 shows an example of microscopic image of fission yeast cells within a distance of 5 mm from the plasma irradiation point after one-day incubation. One of the cells shows elongated shape, which is not seen in wild-type cells under normal conditions. Although only a few percent of plasma-treated cells show such cellular elongation, the phenotype has been highly reproducible. Because the cellular elongation cannot be seen in cells exposed to helium gas only, it is considered that this morphological change was induced by an effect of plasma irradiation.

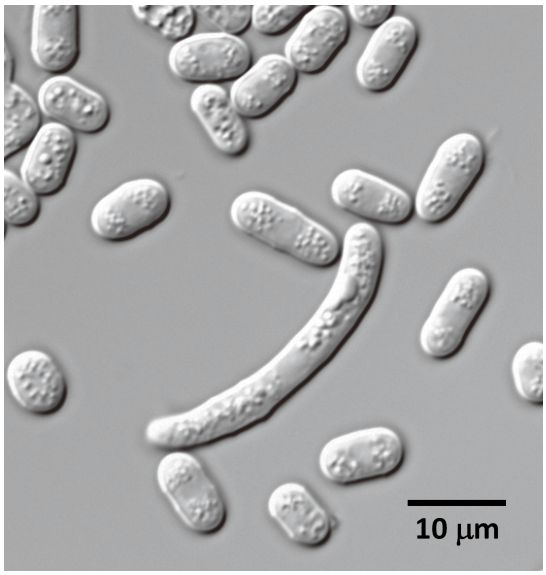

Fig. 2. Microscopic image of fission yeast cells in the proximal of plasma irradiation point. An elongated cell is seen among the normal cells.

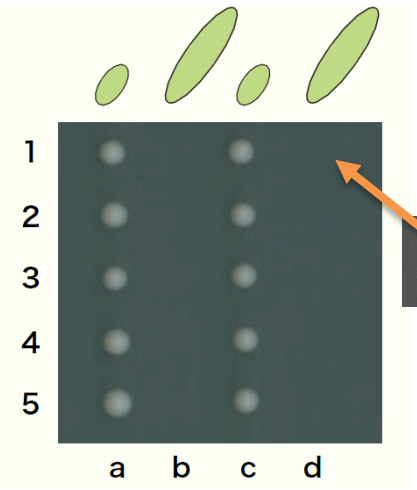

Fig. 3. Normal cells (columns a and c) produced isolated colonies after 4-day incubation on YEA plate, while elongated cells (columns b and d) cannot grow and produce colonies.

It should be pointed out that a similar cell elongation has been reported in dielectric barrier discharge (DBD) plasma treatment of *Bacillus stratosphericus* under wet environment [7], although *Bacillus* are prokaryotes and not eukaryotes like fission yeast *S. pombe*. In addition, cell shrinkage of budding yeast *Saccharomyces cerevisiae* immersed in YPD (yeast extract, peptone, dextrose) media during plasma treatment has also been reported [8].

To investigate whether elongated cells by plasma irradiation could grow, normal cells and elongated cells (ten cells each) were isolated with a micromanipulator and transferred to a new YEA plate, and then incubated for four days at 30 °C. The result is shown in Fig. 3. The normal cells produced isolated colonies; however, the elongated cells was not able to grow. The cellular elongation may be attributable to some abnormality in the cell cycle.

4. Conclusions

We have developed a helium plasma jet system with feeding gas cooling section to perform direct plasma irradiation experiments using fission yeast *S. pombe* under a controlled gas temperature environment. The fission yeast cells on a membrane filter was directly exposed to plasma plume for one minute, where the gas temperature of plasma plume was controlled to 25 °C ± 1˚C. A few percent of the plasma-treated cells showed cellular elongation. The elongated cells cannot grow and produce colonies. It is suggested that cell cycle control may be impaired by plasma exposure.

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