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A micro through-hole chip device for analyzing plasma-irradiation effects on proliferation of cells cultured in liquid media

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Abstract

A micro through-hole chip device was developed for analyzing the effects of plasma-irradiation on cells. The device enables visualization of reactive oxygen and nitrogen species (RONS) behavior in liquid utilizing a pH indicator. Relationship between amounts of RONS produced and proliferation of murine fibroblast cells L929 was analyzed.

1. Introduction

A non-thermal atmospheric pressure plasma (NTAPP) generates reactive oxygen and nitrogen species (RONS) under ambient conditions. NTAPP jet sources have been frequently used in biomedical experiments. One of the typical experimental setup is shown in Fig.1. An NTAPP jet reacts with ambient air generating RONS which are delivered to the liquid medium surface. They diffuse into the liquid medium and reach cells settled on the dish bottom. In biology, RONS have been known to activate or inactivate cells [1-3]. However, it has been difficult to analyze direct plasma-generated RONS effects on cells because the RONS have to diffuse inside the liquid in this configuration. Since the amounts of RONS decrease along the delivery process, the RONS delivery distance should be short in the liquid media.

Previously, we developed a microdevice (*Plasma-on-Chip*) which enables plasma treatment for cells cultured in microwells [4-6]. In the *Plasma-on-Chip* device, the reactive species are delivered to liquid media through gas-liquid interface formed at the microwells. For biological application, *Chlorella* cells were irradiated with NTAPP and fluorescence of the *Chlorella* were found to decrease. However, RONS diffusion in the liquid was not clear. In this study, a micro through-hole chip has been fabricated to analyze the RONS delivery in the liquid media and the RONS effects on cells were investigated.

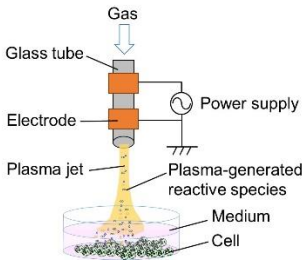

Fig. 1 A typical experimental setup of plasma-irradiation to cells. A NTAPP jet source is used.

2. Experimental Setup

Micro gas-liquid interface for irradiating cells with NTAPP

To deliver RONS to cells cultured in liquid media with a short diffusion distance, a micro gas-liquid interface was used. The principle is shown in Fig.2. A Si chip which has micro through-holes are sandwiched by Teflon holders. When liquid containing cells are poured into a liquid reservoir, the surface tension forms gas-liquid interface at each micro through-hole. The micro through-hole chip is irradiated with NTAPP jet. The RONS are delivered to the gas-liquid interface and reach the cultured cells with a short diffusion distance.

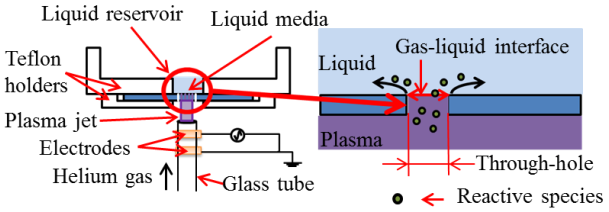

Fig. 2 Cross-sectional illustration of direct RONS supply to cells cultured in liquid.

Fabrication of micro through-hole chip

A micro through-hole chip was fabricated as follows. A thermally oxidized Si substrate (substrate thickness: 200 μm; SiO_2 layer thickness: 3 μm) was used. The substrate was spin-coated with photoresist film and through-holes (50 μm × 50 μm) were patterned by photolithography. The patterned substrate was dipped in HF to etch SiO_2 layer. Then the substrate was transferred to deep reactive etching process (etched depth: 200 μm). The chip was sonicated to brake the microwell bottom forming through-hole as shown in Fig. 3.

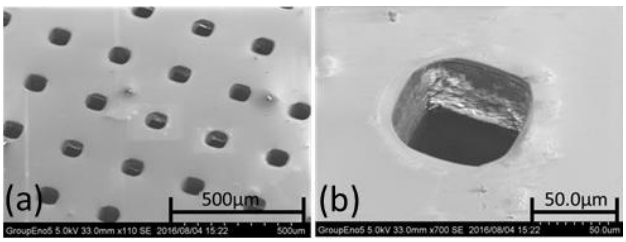

Fig. 3 Scanning electron microscopy images of the through-hole chip. (a) Array of the through-holes. (b) A magnified image.

Visualization of RONS delivery in liquid

When RONS is delivered to liquid, the liquid pH turns acidic. RONS delivery in liquid was visualized using a pH indicator, methyl-red. The solution color turns from yellow to red as the pH changes. During the NTAPP jet exposure, the RONS delivery was monitored by CCD cameras and size of color-changed area was measured.

Analyzing RONS effects on cells

Murine fibroblast cells, L929 was used to analyze the effects of RONS on cells' activities. Cells are cultured in MEM medium containing 10% FCS at 37°C. Cells in liquid medium was set in the reservoir of the Teflon holder. After incubation, NTAPP jet was irradiated to the through-hole device. The morphology change of the cells were observed by differential interference microscopy and the proliferation rate was evaluated.

3. Results and Discussion

Chemically reactive species in He plasma jet

Helium plasma jet was analyzed by optical emission spectroscopy. O and OH radicals which affect activity of a cell were detected. These radicals were derived from oxygen or moisture in the atmosphere.

Size of RONS delivered area

The pH indicator color changed during 120-s plasma-irradiation as shown in Fig. 4. The size of color changed areas were evaluated with varying NTAPP generation voltage and He gas flow rate as shown in Fig. 5. The RONS delivered area ranged from ϕ100 μm to ϕ260 μm.

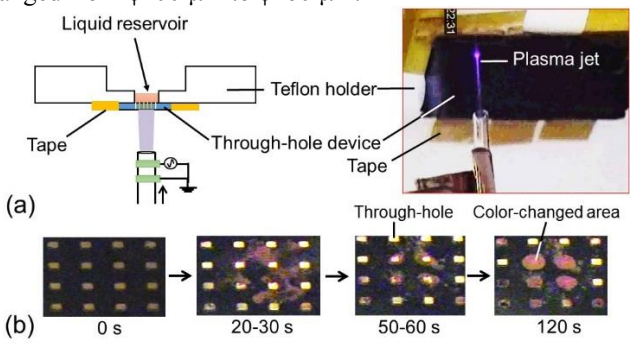

Fig. 4 (a) Irradiating through-hole device from the back side of liquid reservoir. (b) Methyl red solution color changed from yellow (6.2 < pH) to orange (4.4 < pH < 6.2) expanding the area with increasing plasma-irradiation time.

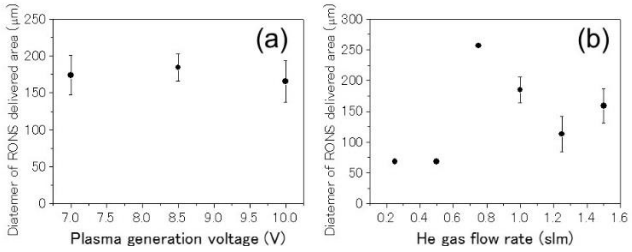

Fig. 5 Diameter of pH changed area as a function of (a) plasma generation voltage and (b) He gas flow rate.

RONS effects on proliferation of L929 cells

After the plasma-irradiation, the L929 cells were incubated for 15 h. Based on the results of the size of RONS delivered area, proliferation rate (rate of cell number after incubation to before incubation) of the cells within ϕ200 μm area was evaluated.

Upon the plasma-irradiation, the proliferation rate decreased from 1.6 to 1.4 [Fig.6(a)]. For comparison, conventional plasma jet configuration as shown in Fig. 1. was also used. The result of 6-s plasma-irradiation with 24-h incubation lead to proliferation rate increase from 2.9 to 3.1 [Fig.6(b)]. The delivery distance from the gas-liquid interface to the cell adhered area were 100 μm and 4 mm for micro through-hole chip setup and conventional plasma jet setup, respectively. The difference in RONS delivery distance in liquid may have caused apparent opposite effects on cell proliferation. It is also interesting to analyze difference in RNA expression in the cells [7]. Further analysis of short-lived RONS (*ex*. O, OH, O_2^-) using micro through-hole chip device will help to understand plasma-irradiation effects on L929 cells.

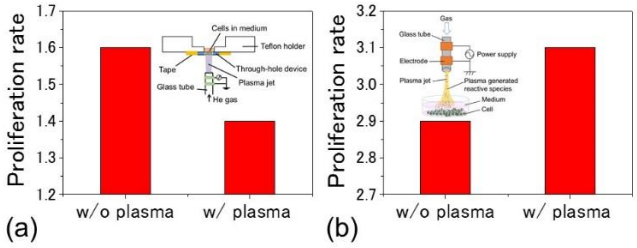

Fig. 6 (a) Proliferation rate under direct plasma-irradiation using the micro through-hole chip. (b) Proliferation rate under conventional plasma-irradiation using the plasma jet source.

Acknowledgements

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