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Role of hydrogen peroxide for inactivation of cell viability by exposure to plasma-treated culture medium

プラズマ処理培地への暴露による細胞不活性化における過酸化水素の役割

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Recently, medical treatments and sterilization using plasma have come to be known as “plasma medicine” since a plasma flow is capable of generating various kinds of stimuli such as chemical species, charged particles, heat, light, shock wave and electric fields. It has been known that a plasma flow has effects on cell viabilities. Here, we focused on an inactivation effect of chemical species generated by a plasma flow and aimed at identifying a key factor of inactivation of cell viability among stable chemical species. To identify the chemical species of the inactivation factor, we focused on the effect of H_2O_2 in plasma-treated culture medium because it is generated in the culture medium and it is also chemically stable compared with free radicals generated by the plasma flow. To elucidate the significance of H_2O_2, we assessed the differences in the effects of plasma-treated medium and H_2O_2-added medium against inactivation of HeLa cell viability. These two media showed comparable effects on HeLa cells and the results supported that among chemical species generated in a plasma-treated culture medium, H_2O_2 is one of main factors responsible for inactivation of HeLa cell viability.

1. Introduction

A plasma flow has been recently applied to plasma medicine since it is easily capable of generating chemically reactive species, light, heat, electric field, and shock wave [1,2]. Plasma inactivation for a low-temperature sterilization [3] have been reported as the outcome of researches in the plasma medicine field.

Since cells/bacteria are generally covered with water, the plasma-water system is one of the key phenomena for understanding the biological response. Various kinds of chemical species, e.g., NO_2, HNO_3, O_3 and OH, generated in air dissolve and transport in water quickly and decreased pH value [4, 5]. However, the mechanism of biological response to plasma flow is still unclear due to its complexity against many stimuli generated.

To identify the specific chemical species which has an inactivation effect on cell viability, we focused on the H_2O_2 among the other species generated by plasma.

2. Experimental methods

The HeLa cell (Institute of Development, Aging and Cancer, Tohoku University) was incubated with a regular culture medium which consists of Minimum Essential Medium with addition of 10% Fetal Bovine Serum and Penicillin-Streptomycin (Penicillin 100u/ml; streptomycin 100μg/ml). The procedure to measure the absorbance intensity which indicates the number of cells was shown in Fig. 1. All incubation were performed at 37 ºC with CO_2 5% for 24 hr. The time-lapse images of the HeLa cell were taken by an inverted microscope

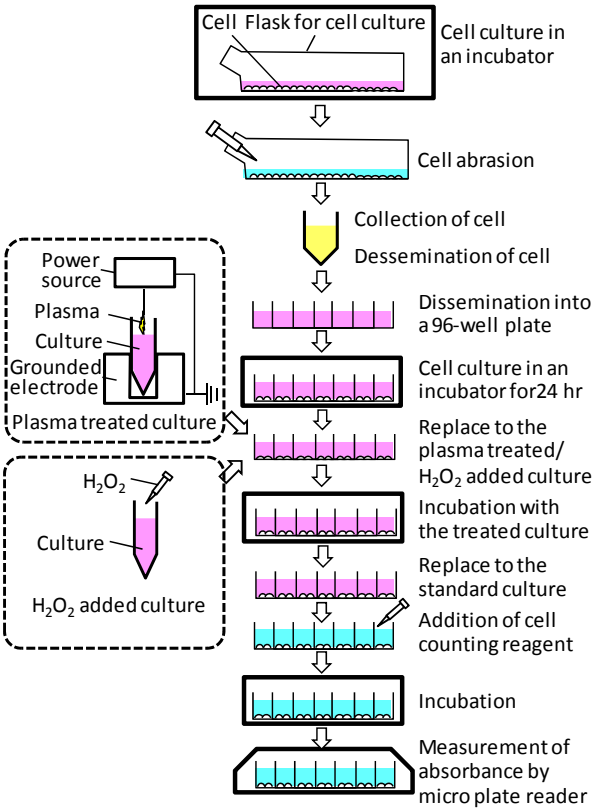

Fig. 1 Experimental setup and procedure.

(Carl Zeiss, Axio Observer D1).

Plasma flow was generated between a needle electrode and the surface of culture medium with a gap of 1.5 mm. The electrode is made of platinum, 0.3 mm in diameter. The culture medium of 1.0 ml in a microtube of 1.5 ml was set in a ground electrode. The voltage of +7.5 kV_{0p} with frequency of 5 kHz and duty ratio of 4% was applied to the needle electrode for 210 s. The power consumption was 7.1 W. The H_2O_2 concentration was 304 μM in the plasma-treated culture medium when the exposure time is 210 s.

3. Experimental results and discussion

Figure 2 shows the cell survival rates shown by the absorption intensities in the cases of regular (control), plasma-treated and H_2O_2-added culture media. The absorption intensities with plasma-treated and H_2O_2-added media decreased with increase of the exposure time and the cells were not alive in the case of 120 min though the control did not decrease. The trend of survival rate against the exposure time for the plasma-treated and H_2O_2-added cases was similar [6].

In order to assess the biological reactions of cells to plasma-treated culture medium, we carried out comprehensive gene expression analysis (Whole Human Genome Microarray Kit, Agilent Technologies) and obtained the comprehensive gene expression profiles. Figure 3 shows the scatter plot of the gene expression intensities made by a GeneSpring software (Agilent Technologies). If the expression intensities of a given gene are the same values between plasma and H_2O_2 cases, the plot is located on the central diagonal line, and if not, it deviates from the line. When gene expression levels in plasma-treated medium were compared with those in H_2O_2-added medium, most of plots for high expression genes were located on the diagonal line, suggesting a comparable cellular response [6].

4. Conclusions

In this study, we have assessed the survival rate and the comprehensive gene expression intensities. It was clarified that H_2O_2 is the inactivation factor of HeLa cell viability among chemical species generated by exposure to the plasma flow.

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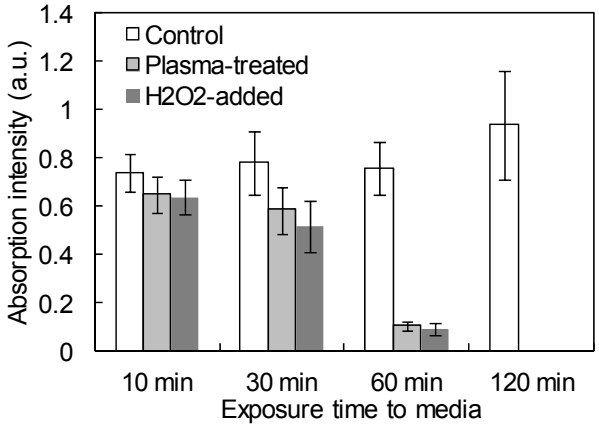

Fig. 2 Cell survival rate shown by the absorption intensity versus exposure time to media of the regular (control), plasma-treated and H_2O_2-added.

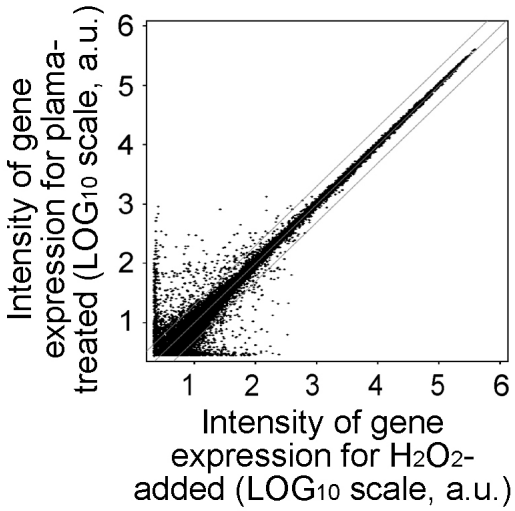

Fig. 3 Scatter plot of the intensities corresponding to each gene under plasma-treated case versus H_2O_2-added case. The values in both vertical and horizontal axes are a common logarithm (Log_{10}).

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