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Effects of in-liquid plasma on enhancement of cell membrane permeability

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1. Introduction

There is a big demand of highly-efficient and minimally-invasive drug introduction technology inside human body to treat many diseases such as cancer, spinal cord injury, and so on. Although the gas-phase atmospheric pressure plasma (APP) is proposed as a promising drug introduction tool [1-8], the accompanying gas flow is not suitable for in-vivo treatment because kind and flow rate of the used gas are limited. To solve this problem, we have tried to apply plasma generated in saline for realizing the highly-efficient drug-introduction inside human body [9].

2. Experimental Apparatus

The experimental setup is shown in Fig. 1. In this work, we generated the in-liquid plasma by applying a pulse voltage (peak voltage : V_{in} = 1.0 ~ 1.5 kV, pulse width : T_{pul} = 100 μs) to coaxial type thin electrode in saline. Here, we directly or indirectly exposed adherent cells to in-liquid plasma. Indirect plasma irradiation (IPI) method was employed to eliminate factors except for products in liquid for a clarification of induced cellular response mechanism. Under IPI, the retention time (t_r) was defined as the time until injection of plasma-generated solution after completion of the plasma generation process at controlled temperature.

The adherent cells was directly or indirectly exposed to the plasma in saline containing drug-simulated fluorescence probe YOYO-1. We evaluated amount of YOYO-1 introduction by measuring intracellular YOYO-1 fluorescence.

3. Experimental Results and Discussion

After the in-liquid plasma irradiation, we observed YOYO-1 fluorescence images using a microscope as shown in Fig.2. YOYO-1 molecules were transferred into the permeabilized cells, and exhibited strong green fluorescence. Significant increase in YOYO-1 fluorescence was observed around the area below high voltage pin-electrode, meaning that in-liquid plasma treatment enhanced the cell membrane permeability. In-liquid plasma is considered to generate flow in solution, ultraviolet (UV) light, electric current and reactive species. Therefore, one(s) of the candidate factors would be responsible for the enhancement of the cell membrane permeability. In this presentation, we will discuss the detailed mechanism of the membrane permeabilization induced by in-liquid plasma irradiation.

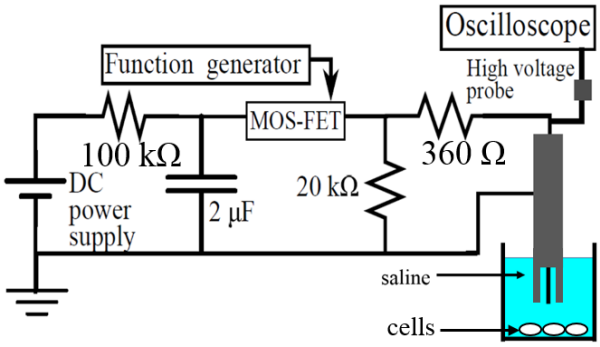

Figure 1. Experimental apparatus for in-liquid plasma.

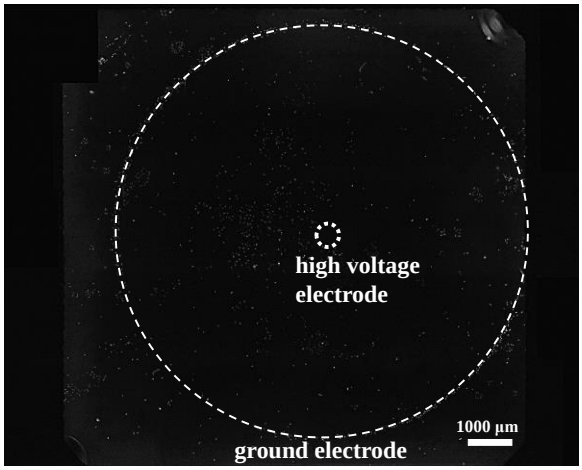

Figure 2. Typical YOYO-1 fluorescence image after in-liquid plasma irradiation treatment.

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