

Gas-Liquid Interfacial Plasmas Enhancing Gene Transfer by Controlling Behavior of Reactive Species

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Gas-liquid interfacial atmospheric-pressure plasma jets (GLI-APPJ) are used medically for plasma-induced gene transfer into cells. In order to realize the minimally-invasive and highly-efficient gene transfer systems, we need to identify the dominant factors, such as UV rays, reactive species, electric field, current, and shock waves, induced by GLI-APPJ responsible for enhancing gene transfer, namely cell-membrane permeability (Fig. 1) [1-6].

Among these factors, we have focused on the plasma-produced reactive species which are classified in terms of their life-span: long-lived (e.g., hydrogen peroxide, H_2O_2), short-lived (e.g., superoxide anion radicals, O_2^-), and extremely-short-lived (e.g., hydroxyl radicals, OH). We have investigated the reactive species in both the gas and liquid phases, focusing particularly on OH radicals, which reportedly play key roles in many medical applications of GLI-APPJ.

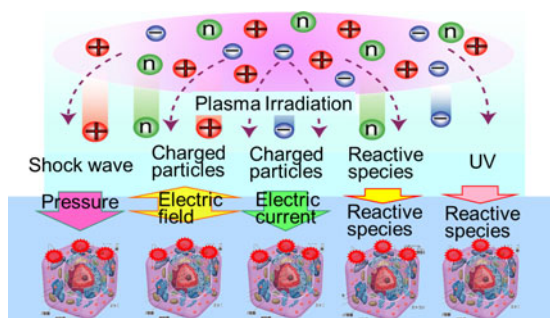


Figure 1. Effects of GLI-APPJ on cell activities.

We generated GLI-APPJ using low frequency (LF) (frequency: 8 - 10 kHz, voltage: 5 - 12 kV) with Helium gas flow, which was exposed to the biological buffer at a controlled thickness (h). To evaluate the spatial mapping of aqueous phase OH radicals (OH_{aq}) and plasma-induced effect on the cell membrane permeabilization, the gelling reagent containing terephthalic acid (TA) and adherent cells (MCF-7 human breast cancer cells) are

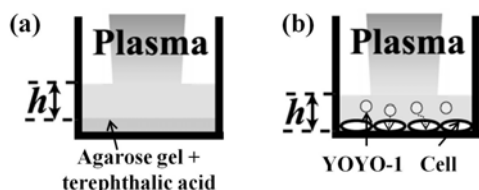


Figure 2. Schematic illustration of the experimental setup for measuring (a) the cell membrane permeability and (b) the horizontal distribution of plasma-produced OH_{aq} using a gelling reagent containing TA.

prepared on 8-well chamber slides, respectively (Fig. 2). Because it is known that plasma-produced OH_{aq} can convert terephthalate anion (which is produced from TA) to 2-hydroxyterephthalate ion (HTA) as a highly fluorescent material, the total production of OH_{aq} can be estimated from the fluorescence intensity. On the other hand, the green fluorescence of YOYO-1 increases 1000-fold when the dye is transferred into cells, which enables us to easily and quickly measure the cell membrane permeability.

Figure 3 shows that fluorescence images of TA-gel (upper) and YOYO-1 (lower) after plasma irradiation. As a result, The concentration of plasma-produced OH_{aq} in the liquid phase region decreases with an increase in solution thickness (<1 mm), and plasma-induced cell-membrane permeabilization is found to decay markedly as the thickness of the solution increases. In addition, the estimated spatial distribution of OH_{aq} corresponded with the distribution of the permeabilized cells by plasma irradiation, which suggests that OH_{aq} play an important role in plasma-induced cell permeabilization.

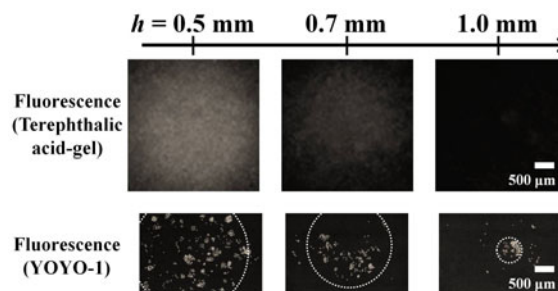


Figure 3. Fluorescence images of TA-gel (upper) and YOYO-1 (lower) after plasma irradiation.

References

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