Drug introduction into cells using direct exposure of gas-liquid interfacial plasmas

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Abstract: Atmospheric pressure plasmas (APP) have recently emerged as a novel tool in drug introduction into cells. However, the method is not suitable for in-vivo treatment due to the accompanying gas flow. Therefore, we tried to apply in-liquid plasma to the drug introduction. Ignition of in-liquid plasma with a first pulse was stabilized by employing pre-treatment. After the stable direct plasma treatment, drug simulated molecules were significantly transferred into cells only below the high voltage electrode. Therefore, in-liquid plasma treatment has potential as new molecule introduction tools.
Keywords: plasma medicine, drug delivery, gas–liquid interface, in-liquid plasma

1. Introduction

There is a big demand of highly-efficient and minimally-invasive drug/gene introduction technology inside human body to treat many diseases such as cancer, spinal cord injury, and so on. However, the conventional drug/gene introduction methods, such as liposome transfection, electroporation, and the viral vector method, have problems in that the introduction efficiency is not very high, the survival fraction is low, and the genes cannot be transferred into some specific lipid cells. Recently, an atmospheric pressure plasma (APP) using helium gas was proposed as a promising drug introduction tool [1-8]. When cultured cells in biological fluids were exposed to the APP, they could be multiply stimulated by shock wave, light, charged particles, and reactive species, which could potentially induce novel cellular response [Fig. 1(a)]. However, the effects of the gas-phase plasma treatment are likely to be ineffective at deep part in liquid and the accompanying gas flow is not suitable for in-vivo treatment because a kind and a flow rate of the used gas are limited. To solve these problems, we have tried to apply plasma generated in saline toward realizing the highly-efficient drug-introduction inside human body [9]. As well as the APP in contact with solution, in-liquid plasma can produce the multiple stimuli and can evoke novel cellular response [Fig. 1(b)].

2. Experimental setup

The experimental schematic for generating in-liquid plasma is shown in Figure 1(c). In this work, the in-liquid plasma was generated by applying a pulse voltage (peak voltage V_{in} = 1.0 ~ 1.5 kV; pulse width T_p = 100 μs) to coaxial type thin electrode which has a curvature radius of less than 100 μm in HEPES-buffered saline (HBS) which has high conductivity. Due to Joule heating and electrolysis, bubbles are generated on the tip and surface of the powered electrode and micro-scale plasma is generated inside the bubble region [Fig.1(d)].

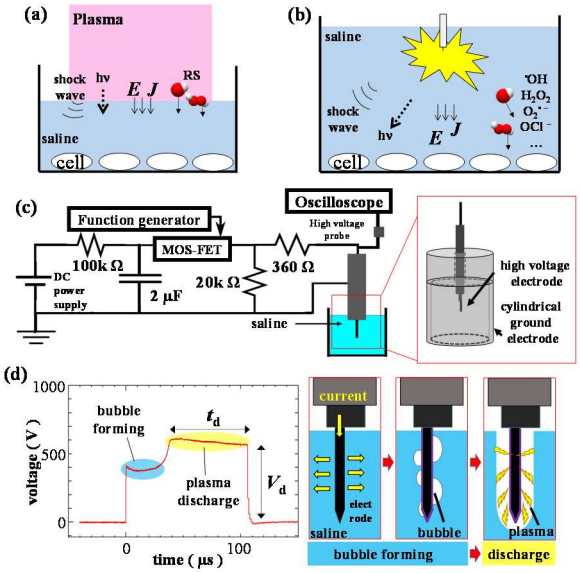

Fig. 1. schematic during irradiation of (a)APP, (b)In-Liquid plasma to cells, (c) Schematic for in-liquid plasma generation and (d)Typical voltage waveform and schematic diagram of in-liquid plasma generation.

In this study, direct treatment method was used. The in-liquid plasma was generated above adherent living cells (mouse fibroblast; 3T3L1) in HBS containing drug-simulated fluorescence molecule YOYO-1 with controlled pulse number (N) at 1 Hz. When YOYO-1, a cell membrane impermeable molecule, reaches at the nucleus into cell membrane, it exhibits strong green fluorescence. Thus, the amount of YOYO-1 transfer corresponds to the YOYO-1 fluorescence. 30 min after the plasma irradiation, YOYO-1 fluorescence images were observed using a fluorescent microscope and YOYO-1 transfer was evaluated as fluorescent area integration.

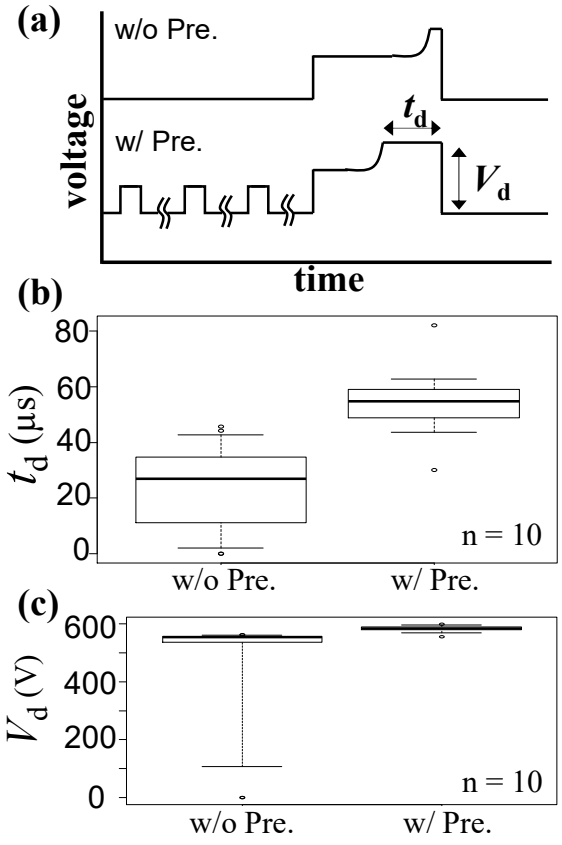

Fig. 2. (a) A schematic drawing of applied voltage waveform without and with pre-treatment, a box plot of (b) discharge time (t_d) and (c) discharge voltage (V_d) without and with the pre-treatment.

3. Results and discussion

As high voltage pulse was repeatedly applied, the generation of in-liquid plasma got stabilized, whereas the changes in the input power were negligible. Therefore, the power in first few pulses seemed to be consumed by other phenomena than the discharge generation. Then, pre-treatment, where relatively low-voltage (0.4 kV) three pulses were applied just before the ignition of the in-liquid plasma, was employed. Figure 2(a) shows the schematic drawing of applied voltage waveform without and with pre-treatment. Figure 2 shows a box plot of (b) discharge time (t_d) and (c) discharge voltage (V_d) without and with the pre-treatment. Pre-treatment decreased variations in t_d and V_d in a first pulse showing that pre-treatment is effective for stable ignition of in-liquid plasma. Then, adherent cells were exposed to the plasma reproducibly.

After the direct exposure of the in-liquid plasma, strong YOYO-1 fluorescence was observed only below the high voltage electrode [Fig.3 (a)], indicating that in-liquid plasma treatment could spatially-selectively enhance the YOYO-1 transfer. As previously noted, in-the liquid plasma can produce the multiple stimuli such as shock wave, light, electric field/current, charged particles, and

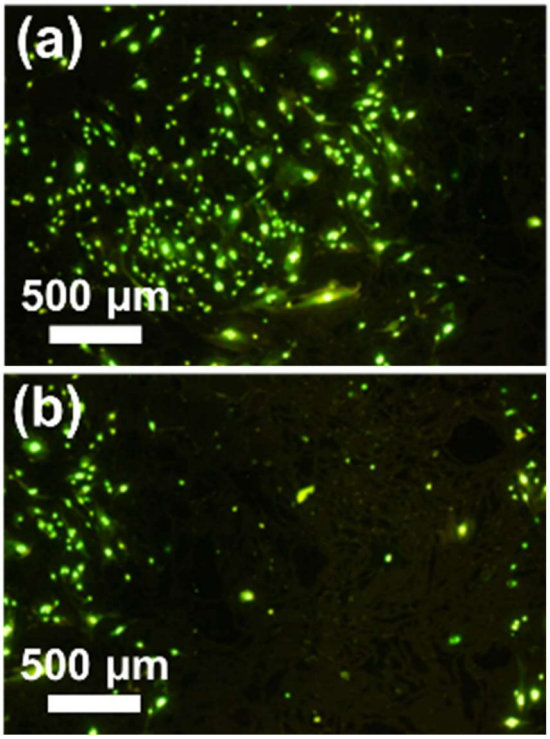

Fig. 3. Top view of YOYO-1 fluorescence (a) below high voltage electrode and (b) other area after the direct exposure of in-liquid plasma (V_{in} = 1.5 kV, N = 5).

reactive species. These stimuli may multiply or synergistically affect the increase in the cell membrane permeability.

In the presentation, the key factors, especially focusing on reactive species, in the drug introduction using in-liquid plasma will be discussed.

4. References

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