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Continuous release of short-lived species induced by plasma irradiation and its application in drug delivery

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1. Introduction

Plasma medicine is a rapidly emerging field, and a number of researchers have reported innovative applications of non-equilibrium atmospheric pressure plasma (APP) and have studied APP-induced cellular responses [1, 2]. While the fact that reactive species are key components of APP in the plasma medical treatment is now widely accepted, the interaction of the reactive species with living cells remains unclear. In such situations, focuses on chemical reaction in APP-exposed solution and cell membrane should be straightforward approaches because first contact of APP-produced reactive species with cells is considered to be just the membrane lipids or the membrane proteins.

Thus, we have ever experimentally shown that unclassified reactive species in APP-exposed solution (organic buffer) can activate transient receptor potential (TRP) channel(s) on cell membrane and induce subsequent cellular uptake of a middle-size membrane-impermeable molecule [3, 4]. The results provide a potential of non-equilibrium APP as a drug delivery tool. However, it is still challenging to specify the key species due to the highly-complicated chemical reaction system in APP-exposed solution. Therefore, we have tried to clarify the chemical reaction system induced by the APP exposure.

2. Experimental Apparatus

Non-equilibrium APP was generated using low frequency (LF) (frequency: 8 - 10 kHz, voltage: 5 - 12 kV) with Helium gas flow. An organic-buffered saline was pre-exposed to the APP and was added to the cultured cells a prescribed time (t_r) after completion of the APP exposure process. The concentrations of H_2O_2 and NO_2^- were obtained using chemical kits based on Trinder's reagent and Griess reagent, respectively. Each chemical probe was added to the APP-exposed saline a prescribed time (t_r) after the APP exposure process.

The treated cell line was mouse fibroblast (3T3-L1). Real-time changes in intracellular calcium ion concentration ($[Ca^{2+}]_i$) and YOYO-1 uptake ($[YOYO]_i$) were obtained using a confocal fluorescent microscope with a intracellular calcium indicator (fluo 4) or membrane-impermeable fluorescent dye (YOYO-1).

3. Experimental Results and Discussion

Figure 1(a) shows the concentrations of hydrogen peroxide (H_2O_2) and nitrite ion (NO_2^-) in the plasma-exposed saline at varying t_r. Both of $[H_2O_2]$ and $[NO_2^-]$ increased but the production rates decreased with increasing t_r (the aging time after APP irradiation), indicating that the APP-exposed saline gradually lost the capacity to produce the precursor of H_2O_2 and NO_2^- over time.

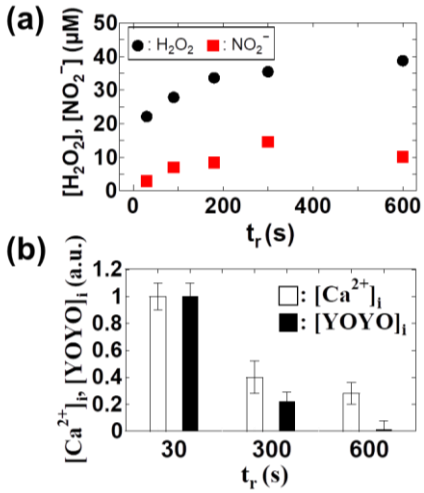

Figure 1. (a) The concentrations of hydrogen peroxide (H_2O_2) and nitrite ion (NO_2^-) in the plasma-exposed saline at varying t_r. (b) Increases in $[Ca^{2+}]_i$ and $[YOYO]_i$ of 3T3-L1 cells stimulated with plasma-exposed saline at t_r = 30 s, 300 s, and 600 s. The mean values ± SE obtained from 23 cells (Ca^{2+}) and 50 cells (YOYO-1) are shown.

Figure 1(b) shows increases in $[Ca^{2+}]_i$ and $[YOYO]_i$ of 3T3-L1 cells stimulated with plasma-exposed buffer at t_r = 30 s, 300 s, and 600 s. Both cellular responses ($[Ca^{2+}]_i$ and $[YOYO]_i$) were significantly lower as t_r increased, indicating that the APP-exposed saline gradually lost the capacity to induce the cellular responses as well. Therefore, the precursor(s) of H_2O_2 and NO_2^- might be responsible for the APP-induced cellular responses.

In the presentation, I will show additional results on the chemical reaction system in the APP-exposed saline and discuss the relation with the evoked cellular responses.

References

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