Experimental study of Plasma-Liquid Interaction
プラズマ照射液相の実験的解析

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1. Introduction

Cancer therapy by indirect plasma-activated medium (PAM) exposure has been reported some effects on anti-proliferative activity against cancer cells such as carcinoma and glioma. [1-3] Although the atmospheric pressure plasma (APP) consists of various active particles such as charged species, neutral species, radicals, energetic photons and so on. Questions how reactive species generated inside the PAM needs to answer.

In this study, we focused on clarification of generation of reactive species such as radicals in the PAM. We have detected electron-spin-resonance (ESR) signals originating generation of radicals in the PAM. From analysis of kinetic behavior of the ESR signals, we will discuss about anti-proliferation effects on the basis of experimental results for chemical modification on the PAM.

2. Experimental

3 ml of cell culture medium (D-MEM+FBS+P/S) in a microplate with 6 well was irradiated by the APP for 3 min. First, HO* radical having highly chemical reactivity was trapped by using spin-trapping agent of 5,5-dimetyl-1-pyrroline-N-oxide (DMPO). Radical densities were analyzed by referencing signal arisen from stable radical; 2,2,6,6-tetramethyl-4-hydroxy piperidine 1-oxyl (TEMPOL).

After the plasma treatment of medium, ESR signals from OH adduct were observed clearly and its concentration was about 11.7 μM. This signal decayed exponentially with reaching the initial value of 1.0 μM for 10 min. The amount of OH adducts and temporal behaviors on the PAM were apparently different compared with plasma activated water activated identically by the APP. Therefore, the set of reactive species involving HO* at least in the PAM may contribute on the effect of anti-proliferation of cancer cells.

3. Results and discussion

After the plasma treatment of the culture medium for 1 min, ESR signals from OH adduct were observed clearly as shown in Figure 1. Quantitatively, concentration of the OH adduct was estimated about 11.7 μM. The observation of OH adduct in water treated plasma was agreed with previous publications [4,5].

The temporal behaviors of signal intensity from the OH adduct were observed as shown in Figure 2. For the culture medium, the signals were rapidly

decayed exponentially with reaching the initial value of 1.0 μM for 10 min. The amount of OH adducts and temporal behaviors on the PAM were apparently different compared with plasma activated water activated identically by the APP.

For water, the generated HO· radical recombined to form hydrogen peroxide (H_2O_2). Indeed, as shown in Figure 2, the decay of OH adduct was approximately 870s and agreed well in literature value [6]. This indicates that HO·+HO·→H_2O_2 reaction dominated. On the other hand, Figure 3 shows H_2O_2 concentration in PAM as a function of plasma exposure time. The H_2O_2 increased linearly with increasing the exposure period. It is noteworthy that absolute concentration of H_2O_2 is relatively small of 1/10 compared with other reports [7-10]. This result of low H_2O_2 might be originated from treatment with contactless of plasma on liquids. Atomic O and N atoms rather than charged species might be effective generate the HO· radical as a source of H_2O_2. Experimentally HO radical generation in culture medium was detected, however no NO radical was observed. Therefore, the set of reactive species involving HO· at least in the PAM may contribute on the effect of anti-proliferation of cancer cells.

4. Summary

We studied about generation mechanism of active species in the cell culture medium by exposing reactive species such as O atoms. The relatively stable species generated in the plasma activated medium (PAM). The concentration of H_2O_2 increased linearly with elapsing of plasma exposure periods with 15.7 μM for 1 min. On the basis of experimental evidence of the generated chemical species, we will discuss the effect of free radicals generated in liquids by the treatments of plasma, related on the biological response under exposure of the plasma activated medium.

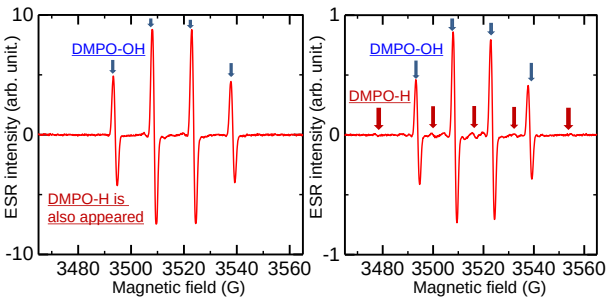

Fig.1. ESR spectra of pure water (left) and culture medium (right) treated plasma for 1 min.

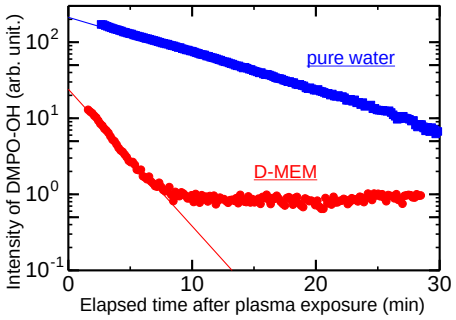

Fig.2. Temporal behavior of signal intensity of DMPO-OH adduct.

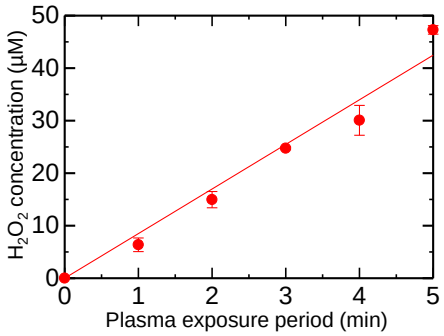

Fig.3. Hydrogen peroxide concentration of PAM

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References

[1] F. Utsumi *et al.*, PLoS one 4 (2013) e81576.
[2] H. Tanaka *et al.*, Plasma Medicine 3 (2013) 265.
[3] S. Iseki *et al.* Appl. Phys. Lett. 100 (2012) 113702.
[4] S. Ikawa *et al.* Plasma Process Polym. 7 (2010) 33.
[5] A. Tani *et al.*, Appl. Phys. Lett. 100 (2012) 254103.
[6] R. Marriott *et al.*, Can. J. Chem. 58 (1980) 803.
[7] T. Sato *et al.*, J. Phys. D: Appl. Phys. 44 (2011) 1.
[8] S. Bekechus *et al.*, Free Radical Res. 48 (2014) 542.
[9] C. van Grils *et al.*, J. Phys. D: Appl. Phys. 46 (2013) 175203.
[10] J. Winter *et al.*, J. Phys. D: Appl. Phys. 47 (2014) 285401.