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Generation mechanism of bactericidal efficacy in the radical-activated water

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Recently, plasma-activated water (PAW) has been paid much attention because of its huge potential for various application forms such as disinfection of medical instruments and water purification. For a decade, many researchers have reported about the mechanism of plasma sterilization in liquid phase and revealed the key species in complicated composition of reactive oxygen and nitrogen species (RONS) in the PAW. [1] The reports indicated short-living species e.g. OH• and HOO• are key species and they also assumed that electrically-neutral radicals are the generators of those species. However, there have been few reports, performing the quantitative measurements of radicals to prove that neutral radicals actually involve in the generation of bactericidal efficacy

In this study, we employed an atmospheric-pressure radical source, which selectively supplies electrically-neutral radicals without charged species or UV photons by extracting them from plasma, to produce radical-activated water (RAW) and evaluated its bactericidal efficacy using the colony forming unit (CFU) of *Escherichia coli* (*E. coli*).

In the experiment, we prepared *E. coli* (10^8/mL) and suspended it into deionized water (DI water). After that, the suspension was treated with the atmospheric-pressure oxygen radical source (Fuji Machine, Tough Plasma) [2], for 0, 1, 3, 5 ,7, 10 min. Then, we collected the samples and dropped 100 μL of them onto NA medium immediately. After being incubated at 37 ºC for 24 hours, we used the colony counting method to investigate the number of survivors in the samples. In addition, we also measured the change of pH values and UV spectra of RAW using UV-Vis-NIR spectrometer (Solid Spec-3700 DUV, Shimazu) and pH meter (S SevenCompactTM pH/Ion, METTLER TOLEDO), respectively. As shown in Fig. 1 , *E. coli* was gradually killed and completely sterilized in the RAW prepared with the radical-irradiation over 7min and the pH values of RAW decreased as the exposure time became longer and reached the minimum of pH 4.33 after 10min-irradiation. As a result of UV absorption spectroscopy, some reactive-nitrogen species (RNS) such as NO_2^- and NO_3^- were also detected as well as reactive-oxygen species (ROS) such as H_2O_2 through the curve-fitting analysis.[3]

By considering the above experimental results, it seems that peroxynitric acid was generated from H_2O_2 and NO_2^- through equation ① - ② and dissociated into HOO• through equation ③, HOO• finally killed *E. coli* in RAW. known as the sterilization factor in the case of PAW, due to the low pH of RAW. [4-5]

$$HNO_2 + H_2O_2 \rightarrow ONOOH + H_2O \quad ①$$

$$ONOOH + H_2O_2 \rightarrow O_2NOOH + H_2O \quad ②$$

$$O_2NOOH \leftrightarrow HOO\bullet + NO_2\bullet \ (pK_a=5.9) \quad ③$$

The question is how RNS such as NO_2^- and NO_3^- was generated in this research although we introduced only O_2 and Ar gases into the radical source. We will discuss about this mechanism in the presentation.

In summary, we investigated the importance of electrically-neutral radicals in plasma on the plasma sterilization in liquid phase and we found that bacteria can be killed even when only radicals are supplied.

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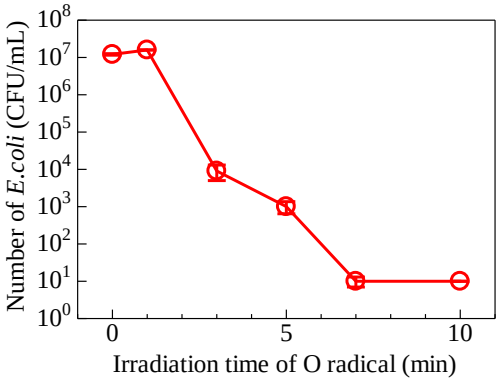

Figure1 shows the result of sterilization using RAW

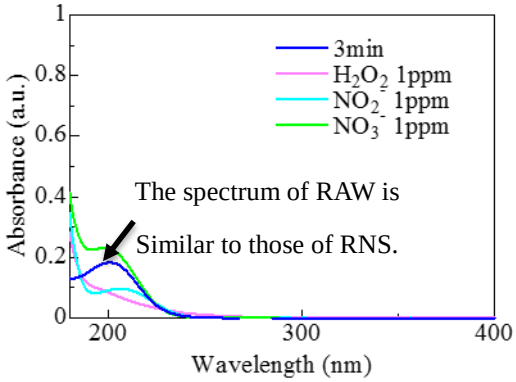

Figure 2 shows the absorbance of 3min-treated RAW and H_2O_2, NO_2^-, NO_3^-.

References

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