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Role of Nitrogen Gas in Plasma-induced Sterilization in Aqueous Solution
プラズマ液中殺菌における窒素ガスの役割

Atsushi Tani[1)], Satoshi Ikawa[2)], Yoichi Nakashima[2)] and Katsuhisa Kitano[3)]

谷　篤史[1)], 井川　聡[2)], 中島　陽一[2)], 北野　勝久[3)]

1)Graduate School of Science, Osaka University
1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan
大阪大学理学研究科　〒560-0043　大阪府豊中市待兼山1-1
2)Technology Research Institution of Osaka Prefecture
2-7-1, Ayumino, Izumi, Osaka 594-1157, Japan
大阪府立産業技術総合研究所 〒594-1157　大阪府和泉市あゆみ野2-7-1
3)CAMT, Graduate School of Engineering, Osaka University
2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan
大阪大学工学研究科　アトミックデザイン研究センター〒565-0871　大阪府吹田市山田丘2-1

Plasma-induced sterilization in aqueous solution has been achieved by reactive species in liquid like oxygen reactive species (ROS) that are induced via gas-liquid interface by plasma or plasma-induced reactive species in gas. Especially, the reduced pH method, where atmospheric pressure plasma is exposed to acidic suspension of microorganisms like bacteria, is characterized by superoxide anion radical ($O_2^-\cdot$) and hydroperoxy radical ($HO_2\cdot$) because the bactericidal activity strongly depends on the pH of the solution. In this paper, we focus on reactive species in plasma-treated water (PTW) prepared by plasma exposure to distilled water. Bactericidal activity in PTW has a lifetime of a few minutes at room temperature and appears only when nitrogen gas was contained in ambient gas and/or the aqueous solution. Nevertheless, formation of $O_2^-\cdot$ is confirmed in the same PTW by electron spin resonance spin-trap measurements. These results suggest that reactive oxygen and nitrogen species as a precursor of $O_2^-\cdot$ are formed in PTW. Nitrogen gas is indispensable to prepare bactericidal PTW.

1. Introduction

Considering medical applications of plasmas, which involve disinfection and wound healing, the supply of active species to solution is extremely important. For plasma disinfection, once atmospheric pressure plasma is exposed to aqueous solution, high bactericidal activity is achieved in the solution via "the reduced pH method" where the solution is sufficiently acidic [1]. Superoxide anion radical ($O_2^-\cdot$) induced in acidic aqueous solutions (lower than pH 4.8) tends to capture a proton (H^+) to form hydroperoxy radical ($HO_2\cdot$), which shows considerably stronger bactericidal activity [2,3]. This indicates that plasma-induced active species in liquid are crucial for medical application of plasmas.

Reactive oxygen species (ROS) including hydroperoxy radical are known to cause damages against nucleic acids, proteins, and lipids. For specific medical applications like plasma disinfection, it is necessary to supply desired active species and simultaneously to avoid supplying unnecessary species to reduce undesirable effects to human body. For this purpose, we investigated formation of ROS including free radicals in water exposed to different types of contact or non-contact atmospheric-pressure helium plasma [4]. In contact plasma, all of those (relatively large amount of $^1O_2$) are induced in the liquid, whereas, in non-contact plasma, $O_2^-\cdot$ can be dominantly induced. Non-contact plasma is one of suitable methods to supply key species for the reduced pH method.

We also investigated formation of ROS in plasma-treated water (PTW), pure water exposed to the plasma. Halflife of bactericidal activity in the PTW strongly depends on temperature (4 sec at 37ºC, a few minutes at room temperature, and several hours at 0ºC) and lower temperature brings longer lifetime. These results reveal that this bactericidal effect is not caused by stable active species like ozone (O_3), hydrogen peroxide (H_2O_2), or nitrogen oxide (NO_x). ESR (electron spin resonance) measurements with spin trapping method and spin oxidation method show intense signal of $O_2^-\cdot$ and no signal of the others like $^1O_2$, O_3, or $OH\cdot$ in the PTW. The activation energy of the $O_2^-\cdot$ signal decay is concordant with that of bactericidal activity. Since high bactericidal activity in PTW is kept by cryopreservation and deactivated soon at body temperature, PTW is another suitable method for plasma disinfection based on the reduced pH method.

Although PTW has such advantages in plasma-induced sterilization in aqueous solution, its mechanisms are unclear at this stage. Since no $O_2^{-}\cdot$ was directly observed in PTW by ESR, another reactive species that can produce $O_2^{-}\cdot$ (hereafter called precursor X) are suspected. In this paper, we investigated the role of nitrogen gas in preparation of bactericidal PTW for physicochemical understanding of the precursor X.

2. Experimental

A low frequency (LF) helium plasma jet was used as a plasma source. PTW was prepared by direct exposure of the LF jet to distilled water in the chamber with ambient gas control, described in the reference [4]. Nitrogen, oxygen, and helium itself were used as ambient gases in the chamber because bactericidal PTW was usually prepared by plasma exposure to water in air. In addition, gas bubbling was performed before PTW preparation to control dissolved gases in water. To detect a short-lived radical species like $O_2^{-}\cdot$, spin trapping reagent, CYPMPO (Radical Research) was used.

3. Results and discussion

ESR signal intensities of $O_2^{-}\cdot$ adduct are shown in Fig. 1. In helium bubbling water (left in Fig. 1), the signal intensities were close to noise level in all samples. In oxygen bubbling water (right in Fig. 1), $O_2^{-}\cdot$ adduct signal was not observed in oxygen ambient gas but in nitrogen ambient gas. In nitrogen bubbling water (middle in Fig. 1), the adduct signal was observed in all ambient gases. Especially, the intense adduct signal was observed in nitrogen bubbling + nitrogen ambient gas condition. Bactericidal activity of the PTW was strong in the same condition.

Once LF plasma is directly exposed to CYPMPO solution in oxygen ambient, obvious $O_2^{-}\cdot$ adduct signal can be detected [4]. However, in PTW, almost no $O_2^{-}\cdot$ adduct signal is observed in oxygen bubbling + oxygen ambient gas condition. It strongly supports that $O_2^{-}\cdot$ in PTW is not directly formed from oxygen but from another reactive species like the precursor X.

To prepare the precursor X in PTW, nitrogen is indispensable, water is possibly necessary, and oxygen is not. This means that the precursor X is a sort of reactive oxygen and nitrogen species (RONS) produced from nitrogen and water. NO and NO_2 do not match to the precursor X because of its short halflife (a few minutes) at room temperature. To identify the precursor X, further analyses are going on.

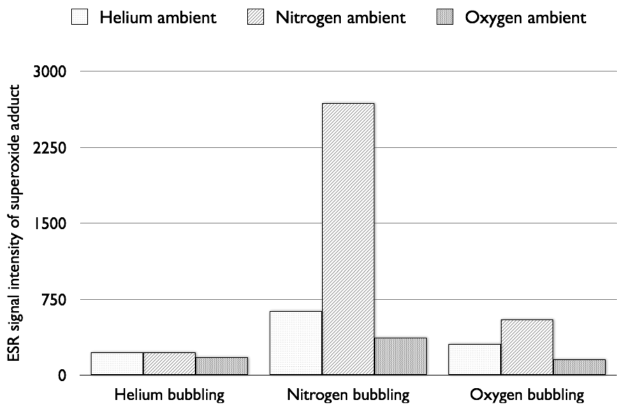

Fig.1. ESR signal intensity of $O_2^{-}\cdot$ adduct in different ambient gases. The initial water samples were prepared by gas bubbling with helium (left), nitrogen (middle), and oxygen (right).

4. Summary

The role of nitrogen gas in preparation of bactericidal PTW was investigated for physicochemical understanding of the precursor X. Nitrogen gas is essential to prepare bactericidal PTW. The precursor X may consist of nitrogen and oxygen like RONS but not be relatively stable NO_x. Further analyses should be necessary to identify the precursor X.

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