Investigation of Reactive Species in Various Gas Plasmas Treated Liquid and Sterilization Effects

Toshihiro Takamatsu¹, Akitsugu Kawate¹, Takaya Oshita¹, Hidekazu Miyahara¹, Akitoshi Okino¹ and Gregory Fridman²

¹Department of Energy Sciences, Tokyo Institute of Technology, Yokohama, Japan
²School of Biomedical Engineering, Drexel University, Philadelphia, USA

Abstract: Conventional atmospheric plasma sources have some limitations in useable plasma gas species, and so biological effect did not be confirmed with various gas plasmas. To generate various gas plasmas we have developed damage-free multi-gas plasma jet source. It can generate stable plasma with various gas species and the plasma does not give thermal damage and electrical shock to the target. The mechanism of bacterial inactivation by plasma irradiation has been thought that reactive species are key factor and the amount of reactive species is different depending on plasma gas composition. In this study, the reactive species generated by various gas plasma and sterilization effect were investigated. As a result, it found that reactive oxygen species, generated by plasma, and low pH value are important factors for effective bacterial inactivation. When the pH was fixed at below 3.6, oxygen plasma could sterilize 6-digits of E. coli in 60 seconds.

Keywords: Atmospheric plasma, Multi-gas plasma, liquid, Sterilization

1. Introduction

In recent years, atmospheric non-thermal plasma attracted attention in medical field because of effective and fast sterilization [1] and wound treatment [2]. However, conventional plasma sources have some limitations in generation of the needed active gas species and the effect of plasma jet in different gas compositions is not well studied. In our group, multi-gas plasma jet source was developed in 2010. This plasma source can generate a stable atmospheric plasma jet with various gas compositions such as helium, argon, oxygen, nitrogen, carbon dioxide, air and their mixtures. And it is free from thermal or electric discharge damage to target materials. In surface treatment using the plasma jet, obvious difference of hydrophilization effect by plasma gas species was observed [3]. So we anticipate that sterilization effect differs according to plasma gas species. The aim of this study is to investigate chemical properties such as pH of plasma treated liquid, reactive oxygen specie (ROS) and reactive nitrogen specie (RNS) generation and their bacterial inactivation effect.

2. Experimental setup

2.1. Atmospheric damage-free multi-gas plasma jet

Experiments were conducted by using damage-free multi-gas plasma jet source of laboratory made. Figure 1 shows the schematic and pictures of the plasma source. The body, 83 mm in length, is grounded and the interior high voltage electrode is connected to an AC power supply [Plasma Concept Tokyo, Inc.] with a frequency of 16 kHz and 9 kV. The generated plasma flows out through a hole 1 mm in diameter at a flow rate of 1 slm. This plasma source can generate stable atmospheric plasma with various gas species such as argon, oxygen, nitrogen, carbon dioxide, air and their mixture gas. The plasma gas temperature was below 57ºC [3].

Fig. 1 Damage-free multi-gas plasma jet.

Fig. 2 Plasma irradiation to liquid in 96 well culture dish.

2.2. Characterization of various gas plasmas

Reactive species are thought as the most important factor for sterilization [4]. Amount of them can be estimated
using photometric measurement of color forming reaction. Nitrogen, oxygen, carbon dioxide, argon and 20% oxygen in nitrogen (simulant dry air gas) were selected as plasma gas. To estimate amount of singlet oxygen, OH radical, nitrite and nitrate generated in each plasma, fluorescence spectrophotometer [LS-55; Perkin Elmer] and UV-VIS spectrometer [U-2910; Hitachi High-Technologies Co.] were used.

The fluorescence by generation of singlet oxygen and OH radical was investigated using solutions of Singlet Oxygen Sensor Green [Invitrogen] and terephthalic acid [Sigma Aldrich], respectively. These reagents were added to 96 well culture dish and were treated directly as shown in Fig. 2. The excitation and emission wavelengths of 504 and 525 nm (as singlet oxygen measurement) and 310 and 425 nm (as OH radical measurement) were observed. Nitrite and nitrate concentration in liquid were measured using Nitrite and Nitrate test kit [HACH]. The nitrite and nitrate in the solution react with these reagents, and each of their absorbance can be measured at 515 nm and 330 nm, respectively.

The pH is also one of the important factors for sterilization [5]. Distilled water was treated at 0 to 160 s with various gas plasmas, and the pH was measured using pH meter [Orion DUAL STAR meter, Thermo scientific].

2.3. Bacterial inactivation effect of various gas plasmas

Inactivation effect on E. coli was investigated using various gas plasmas. E. coli (MG1655) cells with a population of 1x10^6 in distilled water were added to 96 wells culture dish. Argon, oxygen, nitrogen, carbon dioxide and 20% oxygen in nitrogen (as dry air condition) were selected as plasma gas and the bacteria in liquid are irradiated with each gas plasmas. To avoid inactivation effect by ROS, 1% of dimethyl sulfoxide (DMSO), which is an OH radical scavenger, was added and exposed to air plasma for 60 seconds as well as previous setup.

To investigate dependency on pH change, E. coli in 100 µl of citrate buffer that is fixed at various pH levels from 3.2 to 4.8 was irradiated with various gas plasmas for 60 seconds. After the treatment, to operate colony counting, E. coli in the culture dish was distributed to agar medium and was incubated for 16 h at 37°C.

3. Result and discussion

3.1. Reactive species generation

Figure 3 and 4 show fluorescence intensity by singlet oxygen and OH radical in various gas plasmas. As a result, the production of reactive species depends on gas species, and oxygen plasma generated the largest amount of singlet oxygen and OH radical comparing to other gas plasma. The nitrogen plasma could also generate both reactive species. On the other hand, OH radical generation by air plasma was the lowest despite containing oxygen and nitrogen gas.

Figure 5 and 6 show the amount of nitrate and nitrite by various gas plasma treatments. Air plasma could generate nitrite and nitrate around 80 and 38 mg/L respectively, but
their generation by other gas plasma irradiation was below lower detection limit. It indicates that air plasma can generate RNS such as NO and NO\textsubscript{2} in gas phase and the RNS change to nitrite and nitrate by reacting with water.

3.2. pH change by irradiation of various gas plasmas

Fig. 7 shows pH change of distilled water by various gas plasma treatments. Initial pH of distilled water was around 5.3. In case of argon and nitrogen plasma treatment, pH was increased to around 7.0 and 6.0 at 120 seconds, respectively. It is thought that dissolved hydrogen carbonate ion was excluded by flowing gas. In contrast, oxygen, carbon dioxide and air plasma decreased pH to around 4.7, 3.5 and 2.5 at 120 seconds, respectively. In case of oxygen plasma treatment, it suggests that H\textsubscript{2}O change to H\textsuperscript{+}(H\textsubscript{2}O) and OH radical reacting oxygen plasma and H\textsubscript{2}O\textsubscript{2} is immediately created via two OH radicals reaction like (1) to (3).

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\begin{align*}
    O_2 + H_2O \rightarrow H_2O^+ + O_2 & \quad (1) \\
    H_2O^+ + H_2O \rightarrow H^+(H_2O) + OH^- & \quad (2) \\
    OH^- + OH^- \rightarrow H_2O_2 & \quad (3)
\end{align*}
\]

Under carbon dioxide plasma treatment, it is thought that concentration of hydrogen carbonate ion was increased. On the other hand, under air plasma treatment, it indicates that generated nitrite and nitrate in water contribute to pH decreasing.

3.3. Inactivation of \textit{E. coli} by various gas plasmas

Bacterial inactivation effect was studied by plasma treatment of distilled water containing 1x10\textsuperscript{6} of \textit{E. coli}. Fig. 8 shows inactivation effect on \textit{E. coli} in distilled water by various gas plasmas. Among these gas species, air plasma had sterilization effect, and carbon dioxide also can inactivate \textit{E. coli}. However, other gas species such as argon, oxygen and nitrogen had no effect on \textit{E.coli}.

In the case of air plasma treatment, to investigate bacterial inactivation effect of ROS, the distilled water, contained 1% DMSO, was exposed. As a result, it was found that inactivation effect was suppressed by DMSO despite low OH radical generation as shown in Fig. 9.

To investigate bacteria inactivation of pH dependence, \textit{E. coli} in 100 µL of citrate buffer that is fixed at various pH levels was treated with argon, oxygen, nitrogen, carbon dioxide and air gas plasma for 60 seconds. The result
is shown in fig. 10. When pH is 3.2, E. coli was inactivated by all gas compositions used. Specifically, oxygen plasma sterilizes E. coli effectively when pH is below 3.6. It suggests that oxygen radicals, generated by plasma, and pH value are quite important for bacteria inactivation.

4. Summary

The characterization and bacterial inactivation capacity of various gas plasmas were investigated using developed multi-gas plasma jet source. This plasma source generated stable atmospheric plasma with various gas compositions such as argon, oxygen, nitrogen, carbon dioxide and air.

As investigation of reactive species production, amount of singlet oxygen, OH radical, nitrite and nitrate were measured using fluorescence spectrometer and UV-VIS spectrometer. As a result, the amount of reactive species production depended on plasma gas species and oxygen plasma generated the largest amount of singlet oxygen and OH radical in distilled water. On the other hand, air plasma generated the largest amount of nitrite and nitrate in distilled water.

The pH value of plasma treated water also depended on gas species. The 120 seconds of treated water by air plasma was the lowest pH value. In addition, the air plasma sterilized E. coli in distilled water. The inactivation effect was reduced by DMSO that is OH radical scavenger.

When pH was lower, E. coli was inactivated by all gas compositions used. Especially, E. coli was sterilized oxygen plasma effectively when pH was below 3.8. So it is found that low pH and ROS were important factor for sterilization.

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6. References