The Inactivation of Planktonic *Staphylococcus aureus* by Nano-pulsed Argon Plasma Jet Discharge and Its Underlying Mechanism

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Abstract: Outbreaks of waterborne diseases caused by contaminated water can severely threaten human health. Recently, the application of non-thermal plasma for the decontamination of wastewater has been widely studied, but its mechanism is under investigation. This study aimed to investigate the inactivation of *Staphylococcus aureus* (*S. aureus*) and its underlying mechanisms during nano-pulsed plasma jet (n-PPJ) treatment. It was found that a significant reduction of bacteria was started with a sufficient concentration of superoxide radicals (O_2^-•) and hydrogen ions (as pH). All singlet oxygen ($^1O_2$) was produced from O_2^-•, and $^1O_2$ plays a key role in the bactericidal process. Meanwhile, not only was OH• not produced from the decomposition of O_2^-• but it was also observed that it was not involved in bacterial inactivation.

Keywords: Nanosecond-pulsed plasma jet, *Staphylococcus aureus*, Reactive oxygen species (ROS), Inactivation mechanism, Singlet oxygen ($^1O_2$)

1. Instruction

According to World Health Organization (WHO), approximately 3.1 % of all deaths worldwide are attributable to poor water quality [1]. In particular, a variety of severe infections can be caused by the presence of *Staphylococcus aureus* (*S. aureus*). *S. aureus* bacteremia (SAB) caused a mortality rate of 15-25%, higher than infective endocarditis or prosthetic-valve infections [2]. As an emerging method, non-thermal plasma has shown great potential in various applications including water purification, and the food industry. Despite their various application field, their reactions with liquid are under investigation due to the complex reactions between short-lived species (ONOO$^-$/ONOOH, OH•, O_2^-•/HO_2•, and $^1O_2$) and long-lived species (O_3, H_2O_2, NO_2^-/HNO_2, NO_3^-/HNO_3, and H^+). Recently, it is generally accepted that short-lived reactive oxygen species such as OH•, O_2^-•/HO_2•, and $^1O_2$ can induce the death of the cells [3]. Nonetheless, the key species and their generation pathway were not identified clearly. We have studied the characteristics of a single-pin electrode nanosecond-pulsed plasma jet (n-PPJ) as a model plasma device [4]. The interaction between the n-PPJ-induced weakly ionized gas and liquid has been revealed for the last few years. In this work, we performed a bactericidal test by using argon n-PPJ treatment of well-studied Gram-positive bacteria, *S. aureus*, and its inactivation mechanism.

2. Materials and Methods

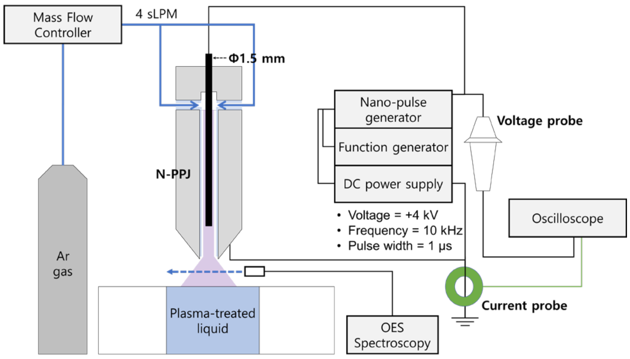

Fig. 1. Schematic of the n-PPJ system.

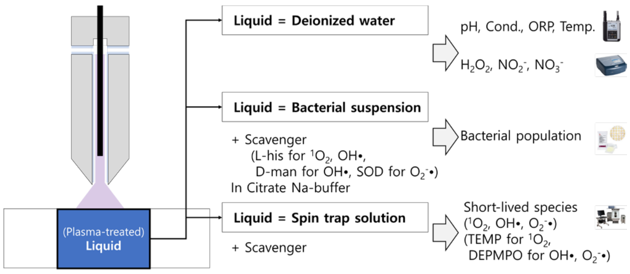

Fig. 2. Experimental methods for each purpose.

3. Results and Discussions

As shown in Fig. 3, the n-PPJ treatment effectively reduces the population of *S. aureus*. More than 6 log reduction was achieved by 10-min of n-PPJ treatment. Meanwhile, the non-linear reduction was observed during the 10-min treatment; the concentration of *S. aureus* was dramatically decreased after 4-min of n-PPJ treatment. As the pH value after 4-min of plasma discharge was approximately 4.8 (pKa of HO_2•/O_2^-•), we established a hypothesis that a dramatic decrease in bacterial population was associated with the presence of HO_2•. (more data such as the concentration of long-lived species and physicochemical properties after n-PPJ treatment will be shown in the Symposium).

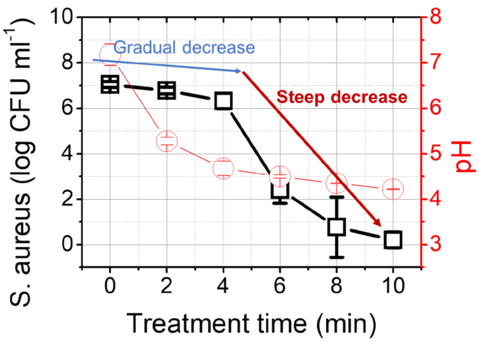

Fig. 3. Typical surviving population of *S. aureus* by n-PPJ treatment

Fig. 4 shows the electrical (applied voltage and discharge current) and optical characteristics of n-PPJ. A typical operation condition of the n-PPJ had a repetition frequency of 10 kHz, and a pulse width of 1 us. pated power during 10-min of n-PPJ treatment did not show a significant difference ($p>0.05$). The optical emission spectrum (OES) ranging from 200 to 1100 nm shows that Ar metastable lines, N_2 second positive system (SPS) ($C^3\Pi_u \rightarrow B^3\Pi_g$), and OH ($A^2\Sigma$ -$X^2\Pi$) band. The electrical and optical signals were not changed in all of the treatment times ($p>0.05$). (data will be presented in ISPC25).

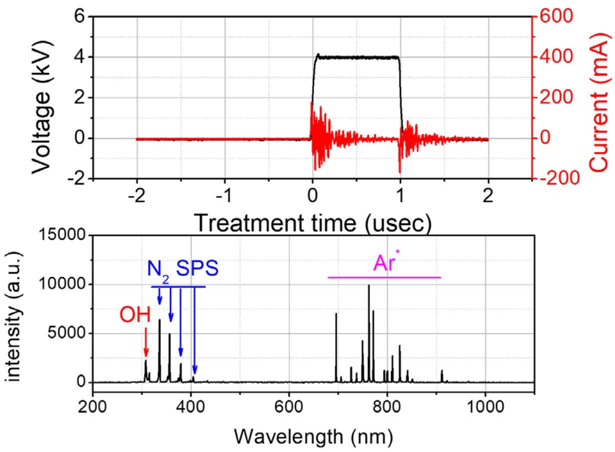

Fig. 4. The electrical (up) and optical (down) characteristics of n-PPJ

Fig. 5 shows that the effect of pH and the activity of O_2^{-}• (as superoxide dismutase; SOD) on the bactericidal efficacy, respectively. Under pH 4.0 maintained using 10 mM citrate-Na buffer, it was clearly shown that microbial reduction was influenced by SOD addition. The microbial reduction was also affected by pH values. A significant reduction was observed when the pH was less than 4.4.

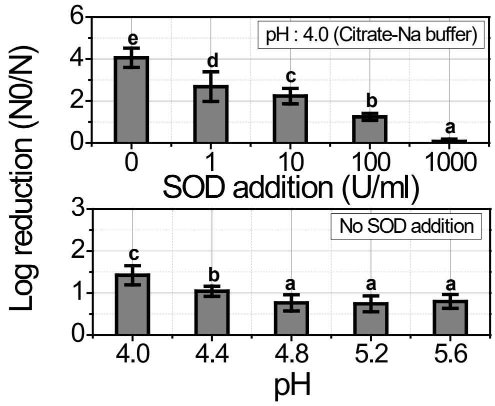

Fig. 5. The effect of [O_2^{-}•] (up) and pH (down) on the population of *S. aureus*

The concentration of $^1O_2$ was affected by the addition of SOD which means that aqueous $^1O_2$ was generated from HO_2• as presented in Fig. 6. Moreover, no $^1O_2$ was found after 1000 U ml^{-1} added sample, indicating that all $^1O_2$ originated from HO_2•. Meanwhile, no significant relationship was observed between the activity of HO_2• and the intensity of OH• (as DEPMPO-OH signal).

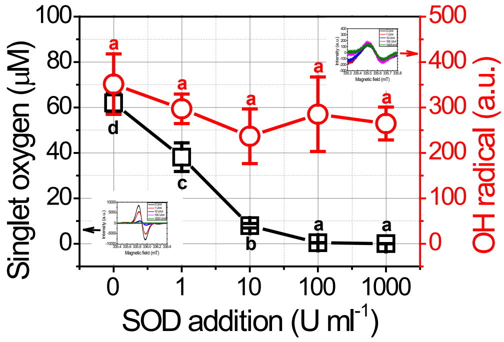

Fig. 6. The effect of SOD addition on the intensity of $^1O_2$ and OH·

A maximum of 1 mM of L-histidine, known as a scavenger of $^1O_2$ and OH• was used in this study due to neutral or alkaline conditions become by an excessive amount of L-his addition. Fig. 7 shows that 1 mM of L-his successfully reduces the $^1O_2$ concentration. Microbial inactivation was significantly neutralized by 0.01, 0.1, and 1 mM L-his addition, respectively, indicating that L-his is strongly associated with the bactericidal process during n-PPJ treatment.

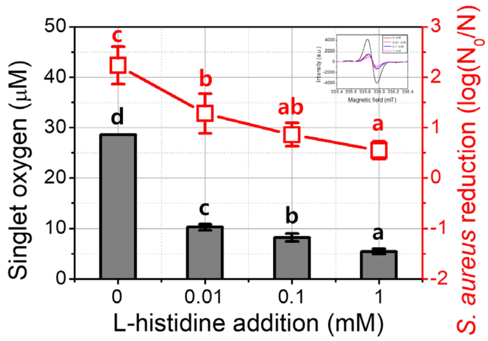

Fig. 7. The effect of L-his addition on the intensity of $^1O_2$ and the population of *S. aureus*

100 mM of D-man which is a well-known OH• scavenger suppresses the activity of OH•. However, 100 mM of D-man was not effective to reduce the population of *S. aureus* for 10-min of treatment, indicating that OH• is not associated with the bactericidal process during n-PPJ discharge.

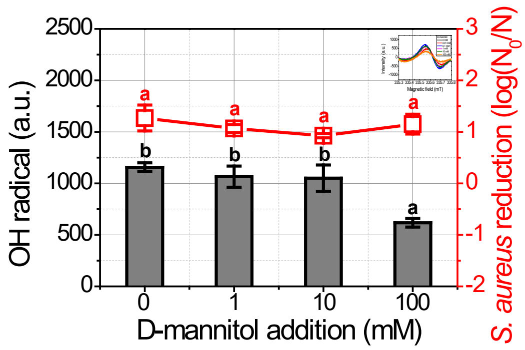

Fig. 7. The effect of D-man addition on the intensity of OH• and the population of *S. aureus*

4. Conclusion

The inactivation mechanism of *S. aureus* in the aqueous phase by n-PPJ was proposed in this study. Firstly, a lot of metastable species including OH, N_2 SPS, and Ar* were generated during Ar-fed n-PPJ discharge. Secondly, short-lived species including OH• and O_2^-•, and long-lived species (H_2O_2, NO_2^-, and NO_3^-) were dissolved in liquid. All $^1O_2$ was generated from HO_2•, and $^1O_2$ was a key species in the antibacterial process. The generation of OH• was not involved in the presence of HO_2•. Moreover, OH• did not participate in the bactericidal process. Our findings can be helpful to enhance the efficiency of plasma devices for the bacterial inactivation process.

5. Acknowledgment

This research was supported by the R&D Program of "Plasma Advanced Technology for Agriculture and Food (Plasma Farming)" through the Korea Institute of Fusion Energy (KFE) funded by the Government funds, Republic of Korea.

6. References

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