Innovative plasma disinfection of bacteria in water by the reduced pH method combined with free radicals supplied by non-contact atmospheric plasma

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Abstract: With the intention of disinfecting human bodies in some medical applications, bacterial inactivation experiments in water have been performed with low-temperature atmospheric-pressure plasmas. We have successfully found that efficient bactericidal activity can be achieved if the solution is sufficiently acidic. There is a critical pH value of about 4.7 for the bactericidal effects, below which the bacteria are efficiently inactivated. The present experiments, where plasmas are indirectly applied to the solution, suggest the importance of highly reactive species generated inside the solution via plasma–air–liquid interactions for the bactericidal effects. It has been also found experimentally that the presence of superoxide anion radicals (O_2^-•) in the solution and the air is essential. The critical pH value may be associated with pKa of the dissociation equilibrium between O_2^-• and hydroperoxy radicals (HOO•), which is known to be approximately 4.8. The formation mechanisms of radicals in solution have been studied from ESR (Electron Spin Resonance) with/without spin trapping agents and MS (Mass Spectroscopy). Air ions of O_2^-• is brought to the water solution to produce O_2^-• in liquid. Bacteria in water can be inactivated by the combination with the reduced pH method and free radicals from non-contact plasma.

Keywords: atmospheric pressure plasma, sterilization, free radical, air ions

1. Introduction

Nonequilibrium atmospheric pressure plasmas have been used in many areas, such as surface modification of polymers. Around at the atmospheric pressure, frequent collisions between neutral gas and high-energy electrons can thermalize plasmas (e.g., arc plasmas) in a very short time scale. However, there are several methods to produce nonthermal (nonequilibrium) plasmas, i.e., plasmas with different electron and neutral temperatures. From a viewpoint of plasma applications to soft materials, such cold plasmas offer attractive opportunities since nonthermal plasmas can supply chemically active species without heating and/or damaging the materials to be processed. In addition, steady-state, uniformly distributed glow-like plasmas are easier to handle for most processes. We have developed the plasma jet system, called as LF plasmajet, which is a system with a single high-voltage electrode and LF (Low Frequency) power supply.

LF plasmajet posses desirable characteristics for plasma processing in the liquid phase due to the highly low-temperature nature of the plasmas and also their flexibility in shape [1]. A liquid can be in general brought to contact with low-temperature plasmas without being evaporated. We have used LF plasmajet to cause various chemical reactions.

2. LF plasmajet generated by a single HV electrode

Engemann et al. have developed an atmospheric pres-sure plasma jet device with a low frequency (LF) power supply in the range of kHz [2]. They used a pair of tubular electrodes is attached to the dielectric tube, through which He gas flows, and connected to the HV power supply (~10kHz, ~10kV). As shown in Fig.1, our system basically uses only a single HV electrode, which is enough to generate plasmas [3]. Discharge occurs between HV electrode and somewhere virtual ground, inside the He gas flux in the air which is brought by the He gas flowing from the tip of the glass tube. Because LF plasmajet contains not short discharge component between electrodes but partial discharge between electrode and faraway ground, the increment of gas temperature is relatively lower than other plasmajet systems with a pair of electrodes. Due to its low gas temperature, as shown in Fig.2, this type of jets is desirable for plasma processing in liquid.

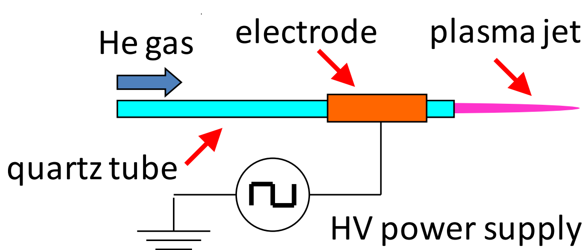

Fig.1 Schematic diagram of LF plasmajet with a sigle High-Voltage electrode.

Fig.2 LF plasmajet exhausted to a finger without burning.

3. Reduced pH method for effective disinfection

With the intention of disinfecting human bodies in some medical applications, bacterial inactivation experiments in aqueous solution have been performed with low-temperature atmospheric-pressure plasmas. We have successfully found that efficient bactericidal activity can be achieved if the solution is sufficiently acidic [4]. The experimental results of plasma inactivation with various pH are shown in Fig.3. It is interesting to note that there is a critical pH value of about 4.7 for the bactericidal effects, below which the bacteria are efficiently inactivated and above which the bacteria are hardly affected by the plasma application. When the plasmas were exposed to 0.5 ml *E.coli* suspensions at pH 6.5, 5.3, 4.7,and 3.8, D values were found to be 19.2, 0.46, 0.27, and 0.08 min., respectively, under our experimental conditions. In fact, drastic bactericidal activity is obtained by controlling the pH of the solution under 4.7. In addition, D value can be controlled to smaller under some condition. We call this technique as the reduced pH method.

From other experiments, it was found that the critical pH value may be associated with pKa of the dissociation equilibrium between superoxide anion radicals (O_2^-•) and hydroperoxy radicals (HOO•), which is known to be approximately 4.8, as shown in Eq. (1).

$$\mathrm{HOO\bullet} \rightleftarrows O_2^-\bullet + H^+ \qquad (1)$$

Although O_2^-• is reasonably reactive, it is considered

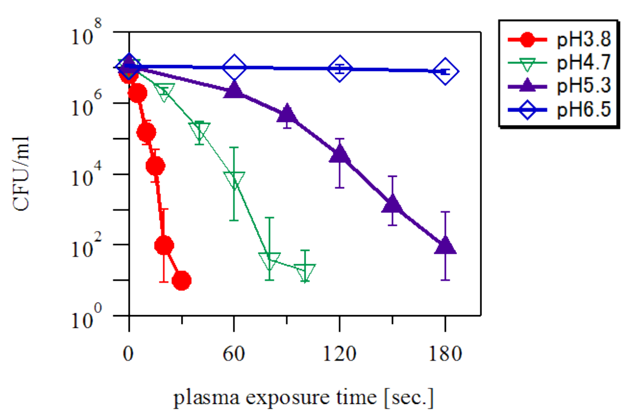

Fig.3 Bacterial inactivation assay in various pH solutions as functions of plasma exposure time.

to be incapable of penetrating the cell membrane due to their electrical charges and therefore unlikely to cause damages inside cells. However, the pH of the solution is sufficiently low, O_2^{-}• is converted to HOO•, which can penetrate the cell membrane and damage intercellular components [5]. This means that the reduced pH method is essential for the inactivation of bacteria in the body fluid, which contains pH buffering ability to control pH 7.4.

4. Superoxide anion radicals in liquid phase by ESR measurement

The formation mechanisms of radicals in liquid have been studied from ESR (Electron Spin Resonance) measurement with/without spin trapping agents[6][7]. It is known that O_2^{-}• in the water solution become extinct by disproportionation reaction and its half life time can be longer in alkaline solution. This means that O_2^{-}• can be trapped in alkali solution. We applied this alkali-trapping method for ESR measurements, instead of spin-trapping method, for high sensitive O_2^{-}• measurements. ESR measurement was done at liquid-nitrogen temperature after the plasma exposure with X-band ESR system (JEOL, JES-FA100).

The plasma exposure experiments were performed against alkali solution (pH14) in the case of non-contact (distance is 20~90mm) with LF plasmajet. This shows the existence of O_2^{-}• with clear signals and the intensity of signal (amount of supplied O_2^{-}•). is inversely proportional to the distance. Unlike the spin-trapping method, the trapping efficiency with the alkali-trapping method is theoretically almost unity. The fact suggests that the existence of some active species in the air.

5. Air ions in gas phase by mass spectroscopy

To clarify the above experimental results, the air 100mm apart from the tip of LF plasmajet was analyzed by MS (Mass Spectrometer) with differential pumping [7]. As seen in Fig.4, the clear

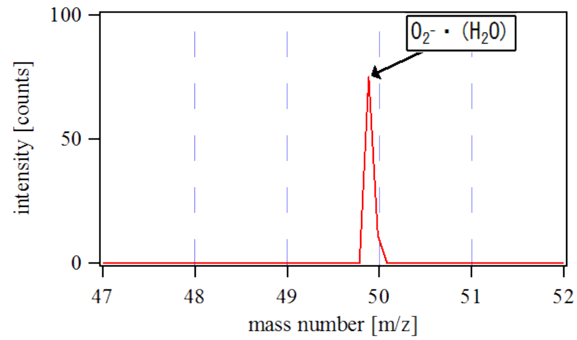

Fig.4 MS spectrum of the air 100mm apart from LF plasmajet.

peak signal of O_2^{-}• hydrated with one water molecule is observed at m/z = 50. This shows the existence of O_2^{-}• in the air. This is a sort of air ion.

6. Inactivation of bacteria in water by non-contact plasma

These experimental results of ESR and MS measurements strongly suggest that the air ions of O_2^{-}• are formed from the air around the LF plasmajet and they are transported into the solution to be O_2^{-}• in water solution. This physicochemical reaction means the disinfection could be possible even by the non-contact plasma where the plasma plume does not touch the surface of liquid. This might bring safety plasma medicine considering the usually used contact plasma to human body.

The experiments were done with contact and non-contact LF plasmajets by changing the distance between LF plasmajet and suspension, and it was confirmed that the disinfection (6 log reduction) was done even in the case with non-contact plasma. Here the interesting experiment was performed by the controlling the O_2 gas mixture. As shown in Fig. 5, LF plasmajets with (a) pure He gas and (b) He gas with 2% O_2 gas mixture were employed. While LF plasmajet with pure He gas was set to be in contact with the surface of the suspension in Fig.5 (a), LF plasmajet with O_2 gas mixture was not to extend outside from the tip of the glass tube in Fig.5 (b). This is a certain type of non-contact plasma. This shortening of the plasma plume extension is brought by impurity of electronegative O_2 gas. As shown in Fig. 6, killing curves was obtained with the pH 4.7

to get the experimental repeatability. Curiously, this shows that non-contact plasma has stronger bactericidal activity than contact plasma in this experimental condition. The most important part of this experiment is that the amount of active species concerning the disinfection supplied to the suspension decides the bactericidal activity and the mixing O_2 gas might bring the increment of the generation of O_2^-• in air and water solution.

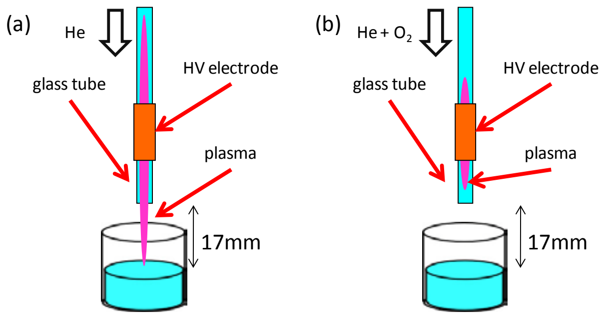

Fig.5 LF plasmajets of (a) pure He gas and (b) He gas mixed with O_2 gas were used to inactivate *E.coli* suspension of 0.5ml. (a) and (b) are corresponding to contact plasma and non-contact plasma, respectively.

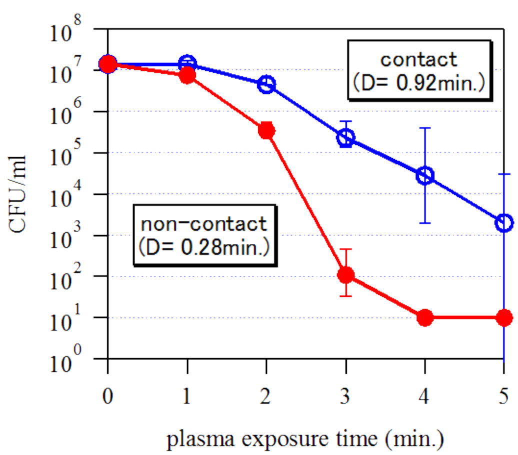

Fig.6 Killing curves by LF plasmajets of pure He gas and He gas mixed with O_2 gas, i.e. by contact plasma (Fig.5 (a)) and non-contact plasma (Fig.5(b)). Detection limit is 10^1 CFU\ml in this procedure.

7. Summary

In concluding, we should note that the disinfection of bacteria in water solution can be achieved with the non-contact plasma which might bring safety plasma medicine considering the usual contact plasma to human body. Key issue is how to supply active species (O_2^-•. in the case with the reduced pH method) efficiently to the solution. Now we are trying to use the reduced pH method to the dental therapy of disinfecting the pulp canal. Many types of oral bacteria were confirmed to be sufficiently inactivated. We believe the reduced pH method is necessary for disinfecting human bodies.

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References

[1] K. Kitano, H. Furusho, Y. Nagasaki, S. Ikawa, and S. Hamaguchi, Proceedings of International Conference on Phenomena in Ionized Gases XXVIII (Prague, July 2007) 2007, 1131.

[2] M. Teschke, J. Kedzierski, E. G. Finantu-Dinu, D. Korzec, and J. Engemann, IEEE Trans. Plasma Sci. 2005, 33, 310.

[3] K. Kitano and S. Hamaguchi, Proc. 18th International Symposium on Plasma Chemistry (ISPC-18) (Kyoto,Japan), 27A-a5, (2007).

[4] S. Ikawa, K. Kitano, S. Hamaguchi, Plasma Process. Polym., 7, 1, pp.33, (2010).

[5]S. S. Korshunov and J. A. Imlay, Mol. Microbiol., 43, 95 (2002).

[6] A. Tani K. Kitano, K. Mizotani, S. Ikawa, S. Hamaguchi, Proc. of Int.Interdisciplinary - Symposium on Gaseous and Liquid Plasmas, (ISGLP, Sep.5-6, Sendai, Japan), p263, (2008).

[7] K Kitano, A. Tani, S. Ikawa, R. Arakawa, N. Ohnishi, International Workshop on Plasmas with Liquids (IWPL2010), 2010/3/23, Hotel Okudougo, Ehime, Japan (2010).