Freezing preservation of the plasma treated water for disinfection treatment in dental and surgical therapies based on the reduced pH method

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Abstract: It has been demonstrated that atmospheric plasmajets can be used for non-conventional plasma processing that involves liquids. For the purpose of disinfecting human bodies in dental and surgical therapies, sterilization experiments in solution have been conducted with low-temperature atmospheric-pressure plasmas. For the plasma disinfection in the body fluid, we have successfully developed the reduced pH method that strong bactericidal activity can be achieved when the solution is sufficiently acidic. In addition, the plasma treated water (PTW) has been found to have strong bactericidal activity for a few minutes at room temperature. Its half lifetime depends on temperature and lower temperature brings longer lifetime. By freezing PTW, it is possible to preserve bactericidal activity for a month at least. We think that the disinfection by unfreezing PTW is a novel method of plasma disinfection. This type of indirect plasma exposure would bring safety plasma disinfection, because the selected supply of active species is possible.

Keywords: plasma medicine, disinfection, sterilization, plasmajet, ROS, free radical

1. Plasma-induced chemical processes in liquid

Based on unique characteristics of atmospheric pressure plasmas, novel plasma processes in liquid have been developed [1]. Various species (ions, electrons, neutrals, radicals, UV, and so on) generated in plasma (gas phase) are used to obtain some desired chemical reactions in liquid phase. For that purpose, atmospheric pressure plasmas with room gas temperature are suitable (Fig. 1). Of course, not plasma itself but only chemical species can penetrate into liquid to induce some chemical reaction. In other words, plasma is a sort of tool for producing reactive species.

For the valuable applications of attractive plasma-induced chemical processes in liquid, many collaborative projects for plasma medicine have been conducted with researchers in different areas (biomaterial [2-4], molecular biology, dentistry, medicine, physical chemistry, biochemistry). Considering plasma medicine, it is important to clarify the elementary processes of the interaction between plasma and living organism. Plasma itself does not directly influence human body and it is important to study plasma induced chemical reactions. For effective and safety applications of plasma to medicine, plasma produced active species must be measured and their reaction to biomacromolecules be evaluated. To clarify the affect of active species to cell, which consists of polysaccharide, amino acid, protein and lipid etc., plasma treated biomacromolecules in liquid were analyzed [5, 6]. The formation of radicals in solution have been studied from ESR (Electron Spin Resonance) and MS (Mass Spectroscopy).

Fig.1 LF plasmajet exposed to a finger without burning.

One of our applications for plasma medicine is the disinfection of human body, such as wound infection. For the medical application to control the infection diseases, inactivation of bacteria in liquid is distinctly important. Considering human body, some reaction should be induced inside body fluid. In gas, bacteria can be directly exposed to plasma and it is not so difficult to inactivate. When bacteria are in liquid, water works as the scavenger of plasma and it generally becomes difficult to inactivate. It is important to say that the technique to kill bacteria in body fluid is required for the plasma disinfection.

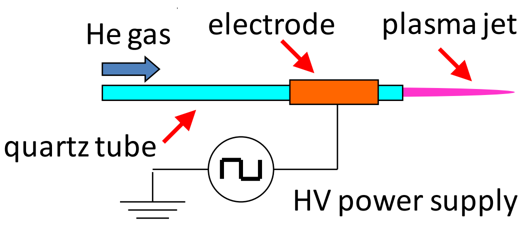

Fig.2 Schematic diagram of LF micro plasmajet with a single electrode.

2. LF plasmajet generated by a single HV electrode

Engemann et al. have developed an atmospheric pressure plasmajet device with a low frequency (LF) power supply in the range of kHz [7]. They and other researchers used a pair of tubular electrodes attached to the dielectric tube, through which He gas flows, and connected to the HV power supply (~10kHz, ~10kV). As shown in Fig.2, our system basically uses only a single HV electrode, which is enough to generate plasmas [8]. 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 usual 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. Diameters of 1 micron ~ 30 mm can be controlled, depending on applications.

3. The reduced pH method for the disinfecting of human body

With the intention of disinfecting human bodies for medical applications, bacterial inactivation experiments in water solution have been done with low-temperature atmospheric-pressure plasmas. We found that drastic bactericidal activity can be achieved if the solution is sufficiently acidic [9].

The experimental results of plasma inactivation with

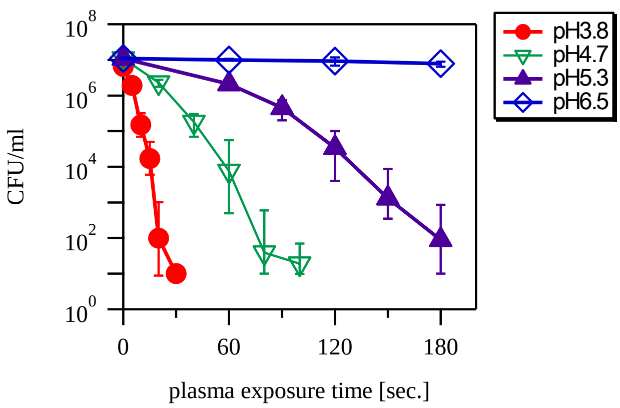

Fig.3 Bacterial inactivation assay in various pH solutions as functions of plasma exposure time.

various pH are shown in Fig.3. LF plasmajet was directly exposed to the bacteria suspension with each pH buffer. 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 (1 log reduction time) 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 (< 2 sec) under some condition. We call this technique as the reduced pH method.

Because body fluid has neutral pH buffer capacity of pH ~7.4, this reduced pH method is essential technique for plasma disinfection. Just before plasma treatment of infected area, acidic fluid should be applied to its surface.

It is considered that strong bactericidal activity is brought by hydroperoxy radical (HOO•) generated from the association of hydrogen ion (H^+) and superoxide anion radical (O_2^-•). The critical pH value is associated with p*K*a of the dissociation equilibrium between these radicals, which is known to be approximately 4.8. This means that O_2^-• can be changed into HOO•, which have much stronger bactericidal activity, in lower pH. Here, long-lived O_2^-• has so much longer half lifetime in water than other reactive oxygen species.

This reduced pH method have been applied to root canal therapy in dentistry. Many kinds of oral pathogens were confirmed to be inactivated efficiently only with lower pH condition [10]. Disinfection experiments inside a root canal of human extracted teeth have been successively achieved. Recently, collaborative research projects of its medical application to bedsore healing, wound healing, surgical site infection have been started.

4. Measurements of radicals in liquid by ESR

The production of O_2^-• in liquid have been confirmed by ESR (Electron Spin Resonance) measurements with/without spin trapping agents[11, 12]. The spin trapping technique is one of methods to observe unstable radicals in solution. Unstable (short lifetime) radicals in liquid are trapped by spin trapping compounds to form spin adducts with a longer lifetime. The sample was aqueous solution of CYPMPO, a spin-trapping compound. ESR measurements were preformed at room temperature with a flat glass cell using the X-band ESR system (JEOL, JES-FA100). Fig. 4 shows a representative ESR spectrum of the solution irradiated directly by plasma. ESR signals for OH• and O_2^-• adducts are observed. It indicates that these reactive oxygen species (ROS) are induced in water. In addition to the experiment of direct plasma treatment, same signals are also observed by indirect plasma treatment with some separation

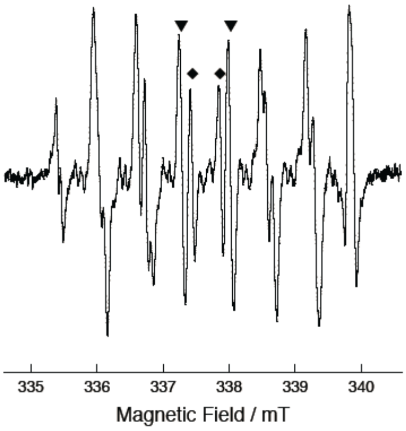

Fig.4 Representative ESR spectrum of the solution. All signals are due to spin adducts of hydroxyl radical (OH·) and superoxide anion radical (O_2^{-}·). The signals can be distinguished using four peaks in the middle (◆ for OH· adduct and ▼ for O_2^{-}· adduct).

distance between plasma and solution. This means that direct plasma exposure is not necessary for ROS supply to solution.

The fact suggests that the existence of some active species in the air. The air apart from 10 cm is analyzed by mass spectrometer (MS) with differential pumping system. The 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.

5. Non-contact plasma disinfection

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.

The disinfection experiment was done with the non-contact plasma with 400 cm extra tube [13]. Afterglow gas of LF plasmajet surrounded by air is exposed to the bacteria suspension. Strong bactericidal activity was seen only when the solution is enough acidic. This means that the reduced pH method is applicable also for non-contact plasma exposure.

Although direct plasma exposure might bring undesired side effects by a wide variety of active species which is unnecessary for bacteria inactivation, indirect plasma treatments by non-contact plasma seem more safety. This might bring safety plasma medicine considering the usually used contact plasma to human body.

6. Plasma treated water (PTW) with the reduced pH method

In addition to the technique for non-contact plasma disinfection of bacteria in liquid, we found that the plasma treated water (PTW) has strong bactericidal activity for a few minutes with the reduced pH method. Pure water exposed to the plasma was incubated for given time period and mixed to bacteria suspension. In other words, the delay time after plasma treatment before mixing bacteria suspension was controlled. As shown in Fig. 5, At < 5 min, 7 log reduction (entirely disinfection) was obtained and around at 15 minutes, there was almost no bactericidal activity. Half lifetime of bactericidal activity was calculated to 2.3 min. Of course, there is the pH dependence with PTW. At neutral pH of 6.8, almost no bacteria inactivation is seen. These results show that this bactericidal effect was not brought by ozone (O_3), hydrogen peroxide (H_2O_2), and/or nitrogen oxide (NO_x). Because their half lifetimes are much longer.

Absolute evaluation of the disinfection power was estimated by mixing the diluted PTW and bacteria suspension (*E. coli*) with acidic condition. As shown in Fig.6, only 0.2% of PTW is enough to bring 5 log reduction of suspension. The result indicates that 0.034% of PTW brings 1 log reduction and undiluted solution (100%) of PTW brings 3,000 log reduction. This extremely strong bactericidal activity of PTW was compared by commercially available antiseptic substance of diluted H_2O_2 by another dilution experiment. Our experimental results showed that undiluted solution (100%) of PTW is corresponding to 105 % of H_2O_2.

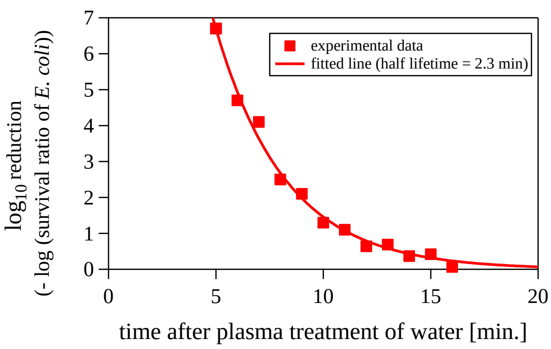

Fig.5 Time decay of bactericidal activity of PTW with the reduced pH method.

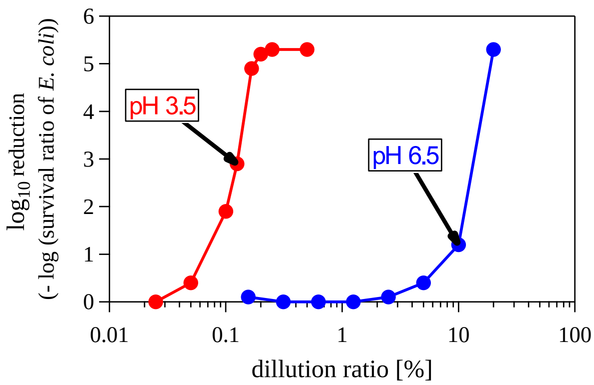

Fig.6 Bactericidal activity of dilution of PTW with the reduced pH method. Detection limit was 5.5 log reduction.

7. Freezing preservation of PTW

Although disinfection experiments described above were done without temperature control (i.e. room temperature), we tried to keep constant temperatures of preserving PTW before mixing bacteria suspension. The half lifetimes were 0.8, 1.8, 2.2, 4.3 min at 25, 20, 19, 15 degree C. Lower temperature brought longer half lifetime. This means PTW can be kept in cold storage.

Further experiments of freezing preservation of PTW were done. After preserving PTW in freezer at some temperature for each duration, PTW was unfreezed to mix bacteria suspension and decays of bactericidal activity were estimated. Half lifetime was 2.9 days, 3 weeks at -18 degree C, -30 degree C. But, by freezing PTW to be preserved at -80 degree C, entirely inactivation of 10^7 CFU/ml suspension was achieved after one month. No decays was seen at -80 degree C. Temperature dependence of half lifetimes is shown in Fig. 7. Amazingly, this was in accordance with Arrhenius equation for PTW both in liquid and solid states. From this estimation, half lifetime at -80 degree C is expected to 700 years.

The indirect plasma disinfection can be done by the combination with PTW and the reduced pH method, which would bring safety plasma medicine considering usual contact or non-contact plasmas for limited supply of required species. PTW can be stably preserved in lower temperature, which makes it easy to handle and use PTW. Now, animal experiments for root canal therapy in dentistry and surgical site infection prevention in surgery have been done with PTW.

8. ESR measurement of PTW

ESR measurements of O_2^-• in liquid were done against PTW. After making PTW, spin trapping agent was mixed. The spin adduct signal of O_2^-• was confirmed in PTW and it decays with time. Its half lifetime was 2.4 minutes at room temperature. Signal of O_2^-• was confirmed in unfreezed PTW kept in freezer. Similar to the disinfection experiment of PTW, temperature dependence of half lifetimes is in accordance with Arrhenius equation. The values of activation energy from bactericidal activity and ESR measurement is almost same.

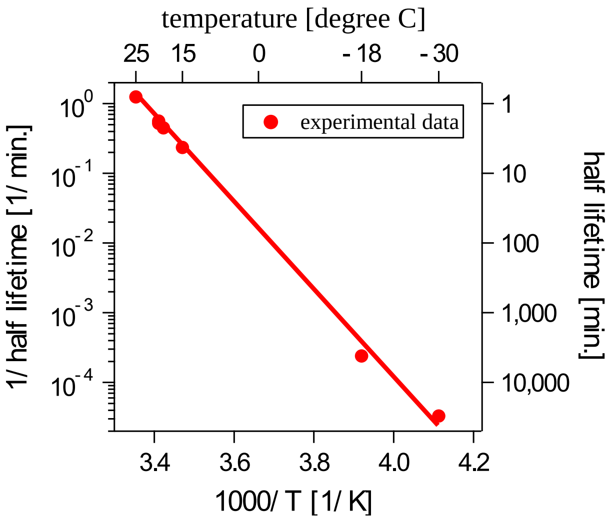

Fig.7 Arrhenius plot of half lifetime of PTW.

Here, half lifetime of O_2^-• is generally known to shorter than that of PTW, which we measured experimentally. No ESR signal was seen against frozen PTW without spin trapping chemical. These facts suggest that some precursor of O_2^-• would exist and its decomposition to generate O_2^-• be in accordance with Arrhenius equation. Components of PTW would not be a sort of commercially available chemical agent, like H_2O_2 or nitrous acid and so on. Further research to analyzing is required.

9. Conclusions

This paper suggests that the indirect plasma disinfection can be done by PTW which contains limited life active species. In that case, plasma is used just as a tool for the synthesis of PTW and there is no need of plasma instruments for disinfection treatment. Freezing preservation of PTW with strong bactericidal activity would bring new era of plasma medicine. It would bring safety plasma medicine considering usual contact or non-contact plasmas to human body.

Acknowledgement

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