Efficiency and Mechanism of Pathogenic bacteria inactivation by Non-Thermal Plasma Activated Water

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Abstract: Four pathogenic bacteria were treated by plasma activated water (PAW) with different duration. The inactivation was evaluated via colony forming unit (CFU) count and fluorescent spectroscope. After PAW treatment 20 min, Effective inactivation of *S. mutans, S. aureus and E. coli* was achieved, while weak inactivation effect to *C. albicans.* It was inferred that hydrogen peroxide and atomic oxygen was the active species in PAW.

Keywords: non-thermal plasma activated water, sterilization, atomic oxygen

1. Introduction

Plasma is regarded as the fourth state of matter, except the solid, liquid and gas state [1]. It is electrically neutral, composed of ions, electrons, free radicals, and chemically reactive neutral particles [2]. In recent years, the non-thermal plasma in the biomedical application has attracted increasing attention, including plasma sterilization [3, 4, 5], wound healing [6, 7], treatment of dental disease [8, 9, 10] and teeth whitening [11, 12, 13]. Especially, Plasma sterilization has become a hot spot in this field. However, plasma micro jet has difficult to reach some tubular instruments (such as catheter and gastroscope tubular) or irregular space because of limited plasma device shape and size. So some of researchers pay close attention to plasma activated water (PAW), which is the water treated by plasma and as the disinfecting solution. Kamgang-Youbi and co-workers have [14] shown that a new disinfecting solution can be obtained by exposing distilled water to gliding arc discharges. Because the radical species present in the glidarc plasma plume [15] (principally ·OH and NO^-) are precursors of other active species in water (NO+, NO^{3-}, and H_2O_2). PAW also has been found to be effective against *Hafnia alvei* [16] as well as *Staphylococcus epidermidis*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae.* The advantage of PAW is its sterilization effect without damage at the same time [17]. Compared with using the plasma jet directly, the dangerous factor of electric current, thermal damage of tissue, and UV irradiation can be avoided by using water-mediated plasma [17]. Furthermore, PAW could reach the cavities or the pipe-like medical equipment easily. So water-mediated plasma expanded the range of plasma application. But seldom research have been done about the disinfection of dental pathogenic bacteria with PAW.

The purpose of this study is to evaluate the PAW inactivation for four pathogenic bacteria (including two pathogenic bacteria of oral diseases) and explore the mechanism.

2. Materials and methods

2.1 Plasma device

The plasma device used in this paper is consisted of two

coaxial copper cylinders as electrodes, which are separated by a dielectric layer with a thickness of around 0.5 mm, the nozzle opening of the plasma device has a diameter of around 0.8 mm. The details of the plasma device and the electrical circuitry can be found in our former study [11] [18]. The sustaining voltage of the plasma micro jet (PMJ) is 450 V, with an operating current of 30 mA. Premixed argon and oxygen gas (volume ratio: 98% Ar and 2% O_2, referred to as Ar/O_2 from here on) was used as the working gas at a flow rate of 5 standard liters per minute (slm).

2.2 PAW preparation

Twenty milliliters of sterile deionized water was treated for 20 min by non-thermal plasma and then used immediately as a disinfectant. The PMJ should under the water level 1 cm (Fig. 1).

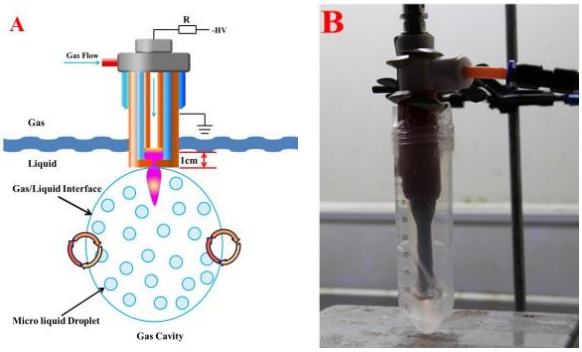

Fig. 1 A: Schematic diagram of PMJ treated deionized water; B: Picture of the PMJ treated deionized water.

2.3 Bacteria Cultures

Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Candida albicans (C. albicans) and *Streptococcus mutans (S. mutans*) were chosen to be treated by PAW. *S. aureus* and *E. coli* were placed in 20 mL Luria–Bertani (LB) liquid and cultured for 12 h under the condition of 180 rpm, 37℃. Then 500 μL bacterial suspension was taken to fresh LB liquid under the condition of 200 rpm and 37℃ activation 1.5 h. *C. albicans* was placed in 30 mL yeast extract peptone dextrose medium (YPD) and cultured for 10 h under the condition of 100 rpm, 37℃. *S. mutans* was placed 1 mL brain heart infusion (BHI) and cultured for 12 h under the condition of 5%CO_2, 37℃. All pathogenic bacteria were reached the logarithmic growth phase.

2.3 PAW treatment and colony forming unit (CFU) count

Each kind of 100 μL cultured bacterial suspensions was added into PAW and the treatment time was 0 min, 5 min, 10 min, 15 min, and 20 min, respectively. The inactivation of bacteria was evaluated via colony forming unit (CFU) count on Petri dish at different PAW treatment time.

2.4 Fluorescence staining

To further verified sterilization effect of PAW, a mixture of SYTO 9 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain propidium iodide were used in this study. The stains differ in their ability to penetrate healthy bacterial cells. Live cells are stained green and dead cells are stained red. For a clearer demonstration of the viability test under fluorescence microscope, higher cell concentrations and longer PAW treatment time was used during this test.

2.5 Hydrogen peroxide detection

A hydrogen peroxide detection kit (Model HYP-1 Cat. No. 22917-00) was used according to the manufacturer's instructions to detect hydrogen peroxide content generated in the PAW.

2.6 Optical Emission Spectroscopy

The contents of the active species in PAW were analyzed with optical emission spectroscopy (OES) using a Multi-Channel Fiber Optic Spectrometer (AvaSpec-2048-8-USB2, Avantes, Eerbeek). The PMJ was submerged in deionized water and one end of the quartz fiber optics cable was placed at the bottom of the water container (quartz tube). The recorded spectra were transferred to a computer for further analysis.

3. Results

3.1 The sterilization effect of PAW

As shown in Fig. 2, when being treated by the PAW for

15 min, the survival of *S. mutans* is 0, *E. coli* and *S. aureus* log value decreased by about 3.6 and 4.When the treatment time prolonged to 20 min, the survival of *E. coli* and *S. aureus* was 0. After 20 min, *C. ablicans* log decline in value of approximately 3. Effective inactivation under PAW treatment of *S. mutans*, *S. aureus*, *E. coli* was achieved within 20 minutes, while weak inactivation effect to *C. albicans.*

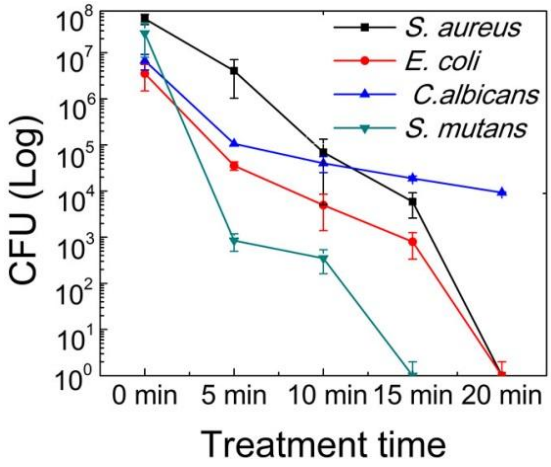

Fig. 2 The sterilization effect of PAW.

3.2 Fluorescence staining result

In the control group (PAW untreated), all bacterial cells were predominantly green .After being treated by PAW for 40 min, *S. mutans*, the majority of *E. coli* and *S. aureus* in the sample were stained red. *C. albicans* cells were still stained green. When the PAW treatment time reached 80 min, *E. coli* and *S. aureus* cell were all stained red, indicating the death of the bacterial cells in the PAW. At the same time, there was part of *C. albicans* dead and the green and red color coexisted.

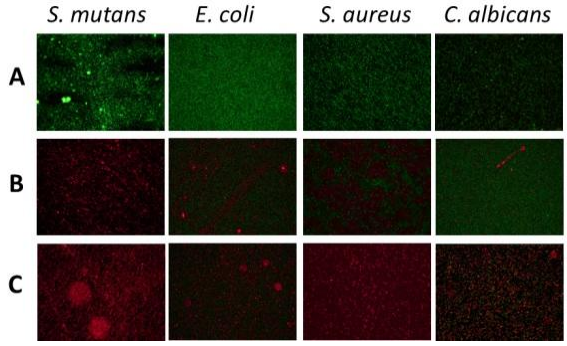

Fig. 3 Fluorescence microscopy images of *S. mutans*, *E.coli*, *S.aureus*, *C. albicans* treated with PAW (live cells are stained green and dead cells are stained red). A: PAW treatment for 0 min; B: PAW treatment for 40 min; C: PAW treatment for 80 min.

3.3 Hydrogen peroxide test result

Figure 4 shows that the content of hydrogen peroxide in the deionized water without being treated by plasma is 0 ppm and that in PAW was about 3.0 ppm.

3.4 OES result

Fig. 5 shows the emission spectrum generated by the Ar/O_2 PMJ operated in water from 200 nm to 820 nm. A strong atomic oxygen emission at 777.2 nm in the spectrum; while the emission spectrum ranging from 706 to 800 nm was dominated by Ar lines.

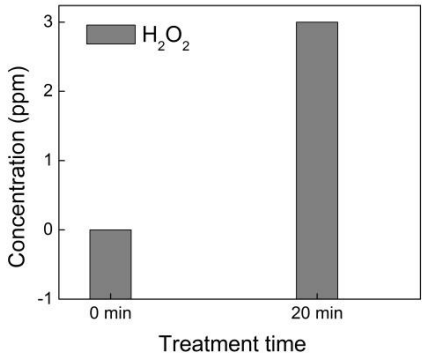

Fig. 4 Detection of H_2O_2 in the deionized water before and after PAW treatment.

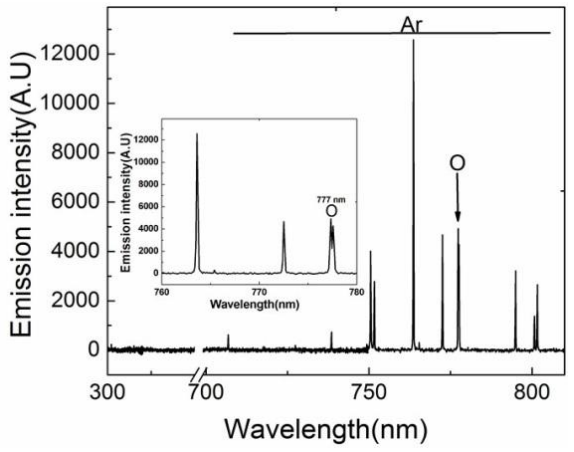

Fig. 5 Optical emission spectrum of Ar/O_2 PMJ operated in water.

References

[1] G. Morfill, New Journal of Physics, 11, 11 (2009).

[2] I. Koban, Journal of Clinical Periodontology, 38, 10 (2011).

[3] M. Laroussi, IEEE Transactions on Plasma Science, 4, 30 (2002).
[4] H. Q. Feng, IEEE Transactions on Plasma Science, 1, 37 (2009).
[5] F. X. Liu, Plasma Processes and Polymers, 7, 3 (2010).
[6] G. Fridman, Plasma Chemistry and Plasma Processing, 4, 26 (2006).
[7] T. Nosenko, New Journal of Physics, 11, 11 (2009).
[8] X. Lu, IEEE Transactions on Plasma Science, 37, 5 (2009).
[9] C. Jiang, Plasma Processes and Polymers, 6, 8 (2009).
[10] J. Pan, Journal of Endodontics, 39, 1 (2012).
[11] P. Sun, IEEE Transactions on Plasma Science, 38, 8 (2010).
[12] H. W. Lee, Journal of Endodontics, 35, 4 (2009).
[13] J. Pan, IEEE Transactions on Plasma Science, 38, 11 (2010).
[14] G. Kamgang-Youbi, Applied Microbiology and Biotechnology, 3, 81 (2008).
[15] B. Benstaali, Plasma Chem Plasma Process, 22, 4 (2002).
[16] G. Kamgang-Youbi, Applied and Environmental Microbiology, 73, 15 (2007).
[17] U. K. Ercan, Plasma Processes and Polymers, 10, 6 (2013).
[18] Q. Zhang, Journal of Applied Physics, 111, 12 (2012).