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Plasma Sterilization Using the High Voltage Pulsed Discharge at Atmospheric Pressure

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Abstract: Rapid, safe and effective sterilization of harmful microorganisms is important for the public in general and hospital patients in particular. Non-equilibrium gaseous discharges have been found to be effective agents for sterilization. The ability to generate these discharges at atmospheric pressure makes the sterilization process practical and inexpensive. Cold plasmas generated by such discharges make their use suitable for applications where medium preservation is desired. In addition, these cold plasmas proved to be very effective due to the synergistic effects of active species, charged particles and UV photons which interact with the cells of microorganisms on the atomic and molecular levels. In this study, a high voltage pulsed plasma sterilization method, a simple and efficient way, was tried on the S*taphylococcus aureus*.

Key words: Atmospheric pressure pulsed plasma, sterilization, microorganisms, *Staphylococcus aureus*

INTRODUCTION

Sterilization is a physical or chemical process that impairs or eliminates microorganisms, especially bacteria. Sterilization of unwanted microorganisms can be achieved by heat, gases and, radiation using electromagnetic or particle (Laroussi, 1996; Rutala, 1996; Moisan *et al*., 2001). Most conventional sterilization techniques are associated with some level of damage to the material or medium supporting the microorganisms. These sterilization techniques require a long process time and create a serious threat for both personnel and the environment (Steelman, 1992; Henn *et al*., 1996). For this reasons, development of new sterilization techniques is extremely important.

Quickly killing of bacteria based on relatively simple, compact and easily transportable equipment is the challenge of present day's researches for the development of new sterilization means. Plasma based techniques are efficient, environmentally sound, fast and cost effective. In order to ensure easy use of such equipment and minimize the cost, researches in this field, are concentrated on the atmospheric pressure plasmas using air as the gas, in which in plasma state, are generating the active bacteria killing elements (Wintenberg *et al*., 1999; Laroussi *et al*., 2000; Purevdorj *et al*., 2002; Feichtinger *et al*., 2003; Moreira *et al*., 2004; Choi *et al*., 2006).

In this study, a high voltage pulsed plasma sterilization method (Ekem *et al*., 2005), a simple and efficient way, was tried on the S*taphylococcus aureus* species. In this method, the bacteria are exposed to the active species of a high voltage (25 kV) pulsed plasma (25 kHz) generated at atmospheric pressure in oxygen, 40 mm above the support of the probes containing bacteria.

MATERIALS AND METHODS

Plasma reactor: The used experimental arrangement is shown schematically in Fig. 1. A hemispherical piece from glass with a radius of 28 mm, is fixed (pressed from upside) on a stainless steel plate via a plastic ring with a diameter of 46 mm. Hemisphere shape glass has an upside tubing through which technical oxygen flux of 0.0023 ltpm is passing. The oxygen exit is provided at the bottom side of the glass hemisphere. Two lateral glass tubing with ϕ = 12 mm are provided on the same line nearly upside of hemisphere. These tubings which are in horizontal position contains the discharge electrodes which are needle shaped tungsten wire electrodes with ϕ = 1 mm. One of the electrodes has only one tungsten wire while the other one has 15 electrodes. All electrodes were sharpened in advance using electro-corrosion in an electrolytic bath for more than 30 min. The thickness of the point of the tungsten wire electrodes

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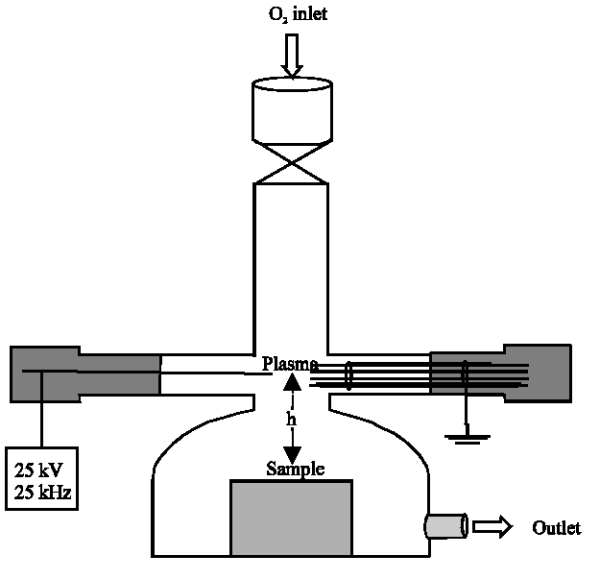

Fig. 1: Schematically view of experimental arrangement

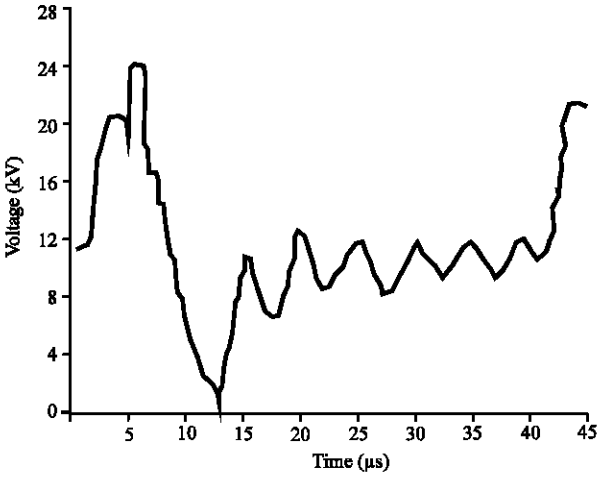

Fig. 2: Oscilloscope image of the high voltage pulsed discharge power supply

were 0.2-0.3 mm. Each electrode was controlled using microscope with a magnification of 500x. The electrodes can be displaced inside of the glass tubing in order to fix needed discharge space between electrodes. On the bottom (center of hemisphere) is a plate on which are positioned samples charged with needed amount of bacteria (which in own case are *Staphylococcus aureus*).

Figure 2 represents the oscilloscope image of the high voltage pulsed discharge power supply. We can see oscillation shape of the applied voltage, clearly. In addition, the peak voltage and frequency of applied voltage calculated by this image.

Following parameters have been considered for the experiment; I; an average current value proportional with the mean pulsed discharge current, h; distance between plasma generating discharge and the cover glass containing the bacteria, d; distance between opposite electrodes (discharge space) and t; total duration time of the discharge, i.e., exposure time of bacteria to plasma.

Because of the needle shaped electrodes, during the half period of the applied voltage pulse, between the electrodes first appear a corona type discharge followed by a transition in time to a streamer type discharge which can not pass to arc because of circuit impedance and also because of the quick decrease of the applied voltage value just after streamer type discharge appearance.

Bacteria: The inactivation effect of the pulsed plasma was tested on the *Staphylococcus aureus* ATCC 2921. Colonies from sheep blood agar (after 18-24 h of growth at 35°C) were picked up with a loop and diluted with 0.9% sterile saline to a turbidity equivalent to that of 0.5 McFarland standard (~10^8 CFU mL^{-1}). Twenty microliter of this suspension was inoculated onto each 10 mm square cover glass which sterilized previously. After inoculation the cover glasses air-dried in sterile plastic petri dishes for 2 h, the cover glasses were transferred to sterile polycarbonate screw-capped tubes and stored until use. One of the cover glasses inoculated with bacteria was kept as control (reference) which not exposed to pulsed plasma. All of the cover glasses except control ones were exposed for t_n min in pulsed plasma. After plasma treatment, cover glasses exposed and control ones were put in 2 mL of phosphate buffered saline containing 0.05% Triton-X 100 and vortexed for 2 min for removal of bacterial cells. After appropriately diluted, 50 μL of each dilution inoculated onto sheep blood agar plates. After overnight incubation at 35°C, blood agar plates were then separately analyzed for colony counts. For all blood agar plates inoculated photo images were taken. For the observation of the morphologies of *S. aureus* cells before and after plasma exposure, cover glasses were stained with Loeffler's methylene blue and light microscopy was performed.

RESULTS

The measuring systems of the experimental arrangement ensure the control of the temperature at the level of the plate, where bacteria are laying during there exposure to plasma. Temperature variation inside the discharge chamber was investigated for various discharge times and discharge currents. The temperature of the support of samples with bacteria exposed to plasma increased during the treatment with only 1-2°C.

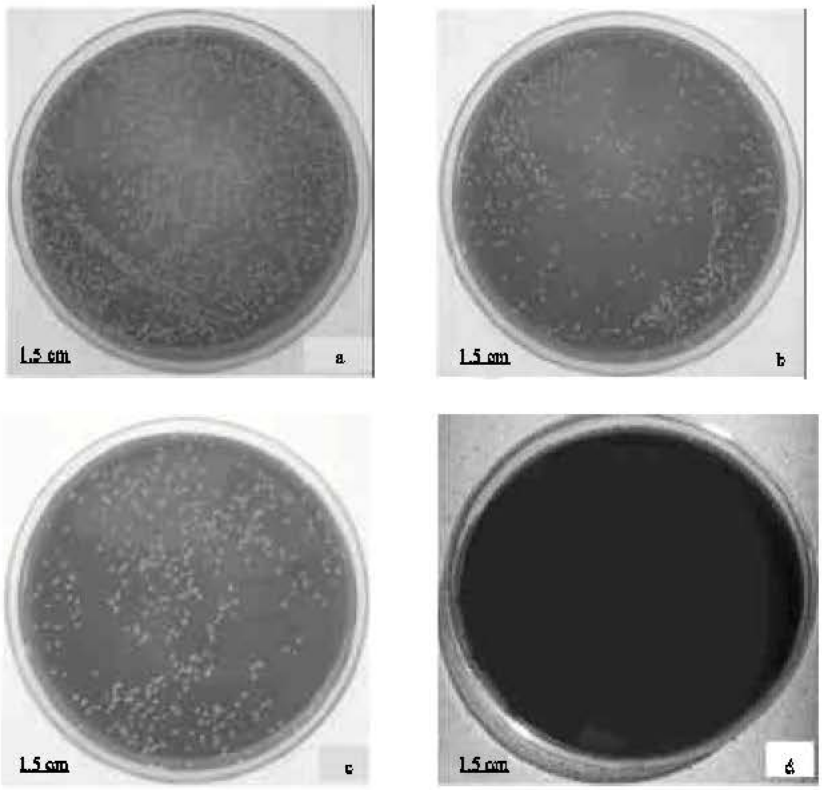

Fig. 3: Appearance of the agar plates after incubation (a) reference plate, (b) 2 min (c) 5 min (d) 12 min exposed to plasma

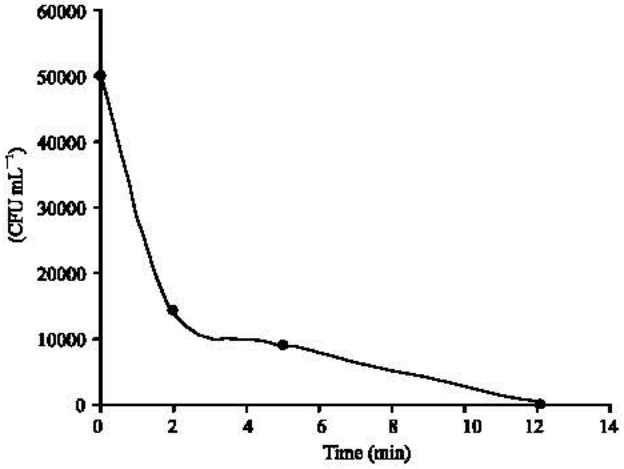

Fig. 4: Number of *S. aureus* surviving versus exposure time

In this study, all experiments for bacteria inactivation using the high voltage pulsed discharge were realized using various different mean discharge currents and h; distance between plasma and the cover glass containing the bacteria and d; distance between electrodes were fixed as 40 and 10 mm, respectively.

Sterilization of *Staphylococcus* bacteria was realized for the mean pulsed discharge current with 40 mA and the number of colonies was counted and we obtained a quantitative result. In Fig. 3, the agar plates after 24 h incubation. The Fig. 3a not exposed to the pulsed plasma treatment contained 50 000 CFU mL^{-1}, while the Fig. 3b and c, exposed to pulsed plasma for 2 and 5 min contained 14 000 and 9 000 CFU mL^{-1}, respectively. The minimum time to kill all bacteria (complete sterilization) on cover glass using 40 mA mean discharge current is 12 min (Fig. 3d). When shorter exposure times than 12 min. performed, for example 2 min. (Fig. 3b) or 5 min (Fig. 3c), the plates still had contained significant amount of bacterial colonies. The quantitative results are given in Fig. 4.

Figure 5 represents the optical microscope images of *Staphylococcus aureus* cells taken at 1000 × magnification by an inverted microscope system installed with polarization optics and digital imaging facilities (Olympus Co. Ltd). Figure 5a corresponds to the reference cover glass while Fig. 5b corresponds to the cover glass exposed to the plasma 10 min for 40 mA discharge current. As shown in Fig. 5 there are a difference in the shape of remaining of the bacteria. These images indicate that the

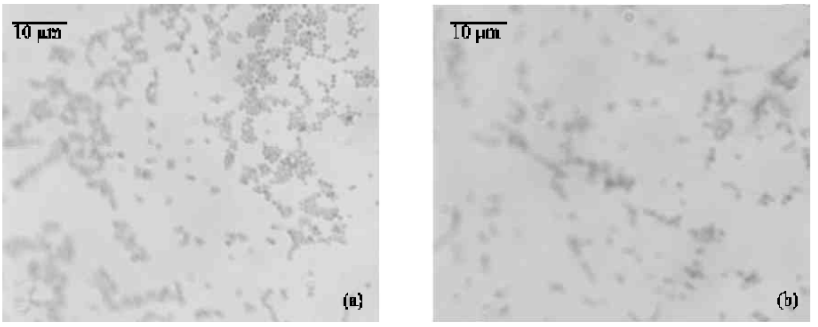

Fig. 5: Optical microscope photo images of *Staphylococcus aureus* (a) before plasma treatment and (b) after 10 min plasma treatment

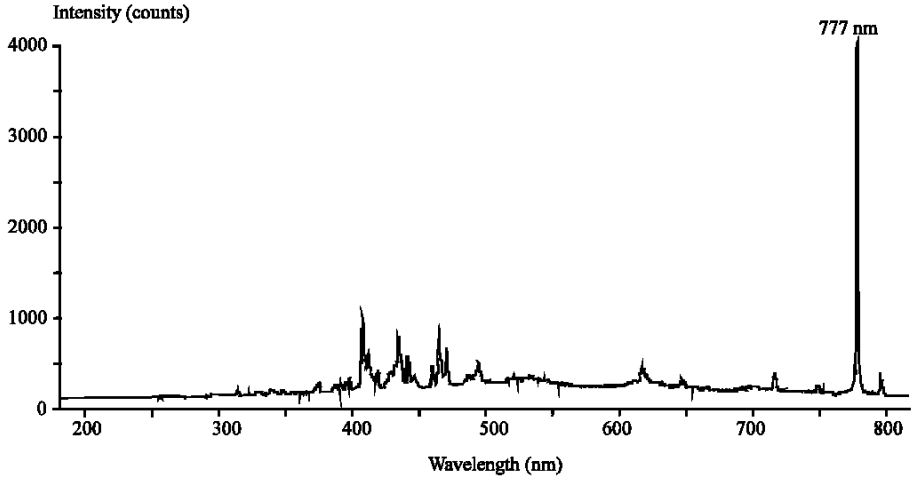

Fig. 6: OES data of pulsed oxygen discharge

high voltage pulsed plasma treatment kills *S. aureus* bacteria. Experimental results show that the sterilization for *S. aureus* bacteria by the high voltage pulsed discharge system can be achieved.

The obtained results show the capacity even of a small pulsed plasma discharge to kill bacteria. The supposed elementary processes taking place in plasma produced by the pulsed high voltage discharge, is described by the following main reactions;

$$e+O_2 \rightarrow e+e+O^+ +O \quad (1)$$

$$O+O_2+M \rightarrow O_3+M \quad (2)$$

$$O^* \rightarrow O+hv \quad (3)$$

Both ozone (O_3) and active oxygen (O) are very reactive species. The last reaction represents the generation of UV light which also contribute to bacteria killing.

We investigated the emission spectrum of oxygen pulsed discharge using Optical Emission Spectrophotometer (OES). Figure 6 shows OES data for the pulsed oxygen discharge. In Fig. 6, we observed the intensive emission spectra of oxygen (777 nm). The existence of intensive atomic oxygen results of sterilization of bacteria.

CONCLUSIONS

The results prove that the high voltage pulsed plasma are able to sterile bacteria effectively. Chemical damage by atomic oxygen and physical damage by ions in the high voltage pulsed plasma are processes for sterilization.

REFERENCES

Choi, J.H., I. Han, H.K. Baik, M.H. Lee, D.W. Han, J.C. Park, I S. Lee, K.M. Song and Y.S. Lim, 2006. Analysis of sterilization effect by pulsed dielectric barrier discharge. J. Electrostat., 64: 17-22.

Ekem, N., Y. Akgün, A. Kiremitçi, T. Akan, S. Pat and G. Musa, 2005. Bacteria killing effect of the pulsed plasma in oxygen+air at atmospheric pressure. Turkish Physical Society 23rd International Physics Congress, Mugla, Turkey, pp: 1369-1372.

Feichtinger, J., A. Schulz, M. Walker and U. Schumacher, 2003. Sterilization with low-pressure microwave plasmas. Surface and Coatings Technol., 174-175: 564-569.

Henn, G.G., C. Birkinshaw, M. Buggy and E. Jones, 1996. A comparison study of effects of gamma-irradition and ethylene oxide< sterilization on the properties of compression moulded poly-D-L-lactide. J. Mat. Sci. Mater. Med., 7: 591-595.

Laroussi, M., 1996. Sterilization of contaminated matter with atmospheric pressure plasma. IEEE Trans. Plasma Sci., 24: 1188-1191.

Laroussi, M., I. Alexeff and W.L. Kang, 2000. Biological decontamination by nonthermal plasmas. IEEE Trans. Plasma Sci., 28: 184-188.

Moisan, M., J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian and L.H. Yahia, 2001. Low-temperature sterilization using gas plasmas: A review of the experiments and an analysis of the inactivation mechanisms. Intl. J. Pharm., 226: 1-21.

Moreira, A.J., R.D. Mansano, T.J.A. Pinto, R. Ruas, L.S. Zambon, M.V. Silva and P.B. Verdonck, 2004. Sterilization by oxygen plasma. Applied Surface Sci., 235: 151-155.

Purevdorj, D., N. Iguru, I. Hayakawa and O. Ariyada, 2002. Inactivation of *Escherichia coli* by microwave induced low temperature argon plasma treatments. J. Food Eng., 53: 341-346.

Rutala, W.A., 1996. Disinfection and sterilization of patient-care items. J. Infect. Control, 17: 377-384.

Steelman, V.M., 1992. Issues in sterilization and disinfection. Urol. Nurs., 12: 123-127.

Wintenberg, K.K., A. Hodge, T.C. Montie, L. Deleanu, D.M. Sherman, J.R. Roth, P.P.Y. Tsai and L.C. Wadsworth, 1999. Use of a one atmosphere uniform glow discharge plasma (OAUGDP) to kill a broad spectrum of microorganisms. J. Vac. Sci. Technol., 17: 1539-1544.