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Sterilization of Tubular Medical Instruments Using Wire-type Dielectric Barrier Discharge

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In this study, sterilization of tubular medical instruments such as catheter and breathing circuit was studied using a wire-type dielectric barrier discharge(DBD) under atmospheric pressure condition. To investigate sterilization factors of DBD under the air condition, we carried out the sterilization experiments using *Geobacillus stearothermophilus* spores under various gas conditions, such as air, 100% N_2 gas, and N_2 and O_2 gas mixtures. It is deduced that the sterilization factors are synergetic effects of UV emission, ozone and OH radicals generated by discharge and/or chemical reaction of water molecule in the air due to UV excitation. In addition, we demonstrated a twisted wire-type DBD using two copper wires with diameter of 0.2mm by applying a voltage of ±0.75kV at a frequency of 5 kHz. Using the wire-type DBD, we confirmed the discharge inside resin tube for the medical with inner diameter of 4mm and length of 80cm.

Keywords: Dielectric barrier discharge, Water vapor, Ozone, Ultraviolet emission, OH radicals, Atmospheric presssure

1. Introduction

Sterilization is generally demanded for hygiene management in the medical and food industries. Various kinds of sterilization methods, such as autoclave, gamma-ray irradiation, dry heat or moist-heat sterilization, ethylene-oxide gas (EOG) sterilization and so on, are widely used depending on the materials to be sterilized. However, there are various concerns regarding these conventional sterilization methods. For instance, dry-heat or moist-heat sterilization techniques require the use of heat-resistant and/or wet-resistant materials such as metal or glass implements. On the other hand, EOG sterilization can be used at low temperature and is suitable for the complicated structure, but EOG is very toxic and thus environmentally unsuitable. Furthermore, it is needed a long time after sterilization process, as aeration time for reduced the toxic ethylene-oxide residuals to safety level. Thus low-temperature sterilization techniques using low pressure and atmospheric pressure plasma have been extensively studied as one of alternative sterilization methods. [1]-[5] However, sterilization experiments using the complicated medical instruments or medical goods wrapped by specific package are very few, to our knowledge.

In the present study, we aimed at developing the low temperature sterilization technique using a wire-shape DBD for medical instruments with thin tube structures, such as heat-sensitive catheters or breathing tubes.

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2. Experimental Set-up

Figure 1 shows a schematic drawing of the experimental setup for atmospheric pressure sterilization using linear coaxial DBD electrodes.[6] The coaxial electrodes consisted of a quarts tube with diameter of 3mm and length of 25.5 cm as a dielectric layer and tungsten wire and aluminum tape as inner and outer electrodes. The linear DBD plasma was generated by applying a voltage of ±4 kV at frequency of 5 kHz.

For the internal sterilization experiment, we set the linear coaxial DBD electrodes and the biological indicator inside the resin tube. Here, we used the biological indicators (BIs) of *Geobacillus stearothermophilus* (ATCCNo.12980) with a population of 3.0 × 10^6, deposited on an oblong polished stainless steel disc. After plasma irradiation, the BI samples was incubated in a culture tube with tryptic soy broth for seven days or more, at an incubation temperature of 55~60°C. Then we can determine whether the spores were inactivated or not, by confirming that the color of the tryptic soy broth kept the original purple color when the spores were perfectly killed or changed to yellow when the spores were still viable.

To study the sterilizing factor of the present linear-shape DBD at air circumstance, we used the air (room temperature of 27 ºC and humidity of 67%RH), N_2 gas, and two types of N_2/O_2 mixture gas ($N_2:O_2$ =50:50 and 80:20 as the dry air-simulated gas). We carried out

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the optical emission measurements using the UV-visible spectrometer (Acton, SpectraPro 2300i) having the wavelength range of 200~800 nm. In addition we measured ozone concentration in the chamber. We also analyzed the morphology of the spores after the plasma irradiation, using the scanning electron microscope(SEM) (JEOL, JSM-6360).

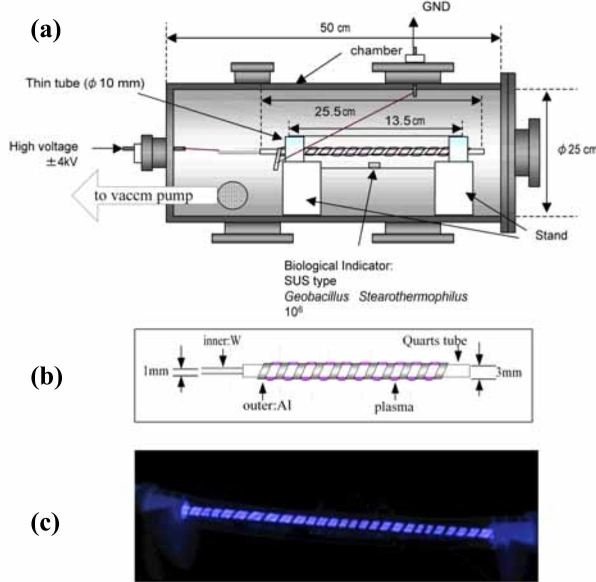


Fig.1 Schematic drawing of experimental setup; (a) whole experimental setup, (b) schematic drawing of linear coaxial DBD electrodes, and (c) a photograph of discharge of a linear DBD electrodes.

3. Results and discussion

The sterilization experiments were carried out by using the linear DBD electrodes set inside the resin tube. To change the surrounding gas, we carried out the sterilization experiment inside the stainless steel chamber, with a diameter of 25cm and a length of 50cm. The N_2 gas or N_2/O_2 mixture gas was introduced into the chamber after pumping by a rotary pump. The DBD plasma was generated by applying ac voltage of 4 kV at a frequency of 5 kHz during during the plasma irradiation time up to 5~60 min. Results of inner sterilization experiment of BIs under various gas conditions are tabulated in Table 1.

Table 1 Results of inner sterilization experiment using several gases. (+:not inactivated -:inactivated No Data: no experiment)

	Plasma irradiation time [min]						
Gas species	5	10	20	30	40	50	60
Air	+	–	–	–	–	–	–
N_2:O_2(50:50)	+	+	+	No data	+	+	+
N_2:O_2(80:20)	+	+	+	+	+	+	+
N_2	No data	+	+	+	+	+	+

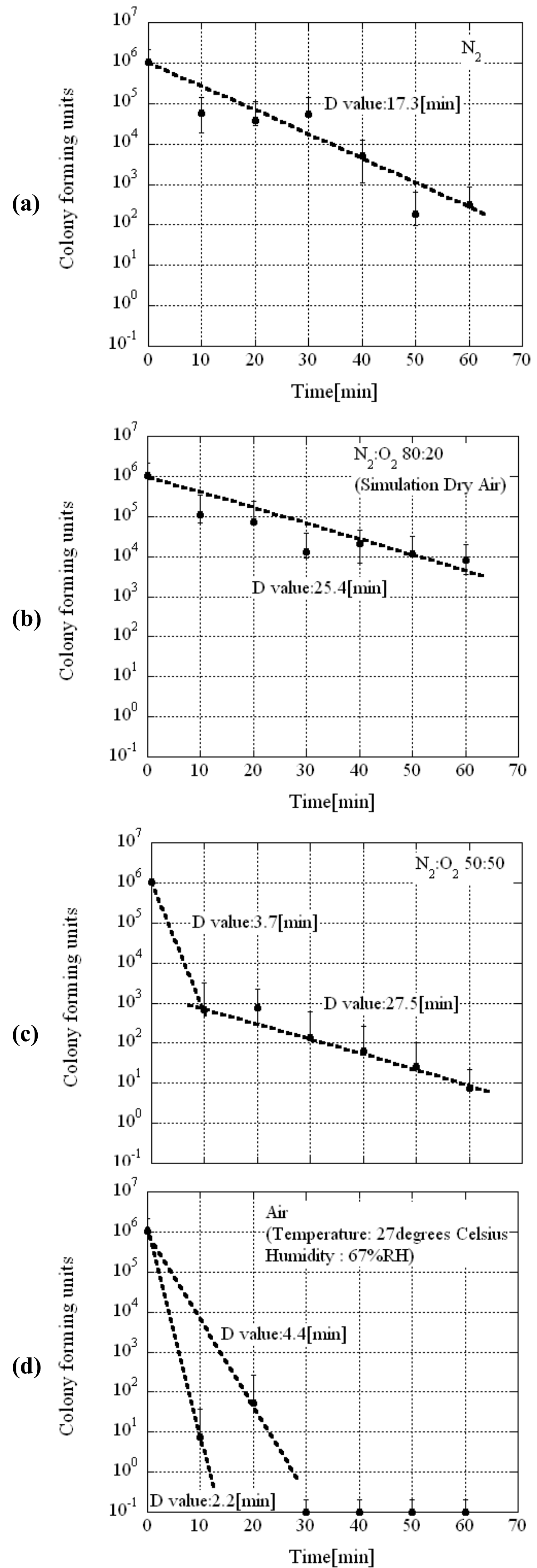


Fig. 2 Results of colony count measurement using BIs set inside the resin tube under different gas conditions.

In Table 1, the signs of "+" and "–" mean that spores were "not inactivated" and "inactivated", respectively. No data means that we have not performed the sterilization test. To evaluate the killing rate for various gas conditions, we used the colony counting method. The plasma irradiation time was varied up to 60 min. and we used *G. stearothermophilus* spores of SUS type BIs.

After plasma irradiation, spores were taken off from the SUS disc and mixed with culture medium, then some amount of culture medium was pasted over the agar medium of the Petri dish. The numbers of colony forming units were then counted and results are shown in Fig. 2. From the decaying slope of spore numbers, we can evaluate the D-value which is defined as the time taken to reduce the surviving spores by one order. In present cases, we obtain the D-values of 17.3min in the case of 100% N_2(see Fig. 2(a)), 25.4min in the case of N_2:O_2 of 80:20(Fig. 2(b)), two slopes with 3.7min and 27.5min in the case of N_2:O_2 of 50:50(Fig. 2(c)), and 2.2min to 4.4min in the case of actual moist air (Fig. 2(d)), respectively.

In order to investigate the characteristic of UV emission radiated from the DBD plasma under atmospheric pressure, we have carried out the optical emission spectrometry (OES). Figure 3 shows the emission spectra of DBD plasma under various gas conditions described above. It is seen that the emission of second positive system of the nitrogen molecules is predominant for all cases.

These spectral intensities apparently increase with an increase of N_2 mixture ratio. This is a reason why we observed smaller D-value in the case of 100% N_2 gas condition than air-simulated N_2/O_2 gas mixture. It is noted here that the observed emission spectrum is very analogous to those in the case of low-pressure nitrogen microwave plasma[7], where a population of 10^6 *G. stearothermophilus* spores were killed after 5 min of plasma irradiation [8].

We also carried out the measurement of ozone concentration inside the chamber using ozone sensor (ECO SENSORS, INC. OZONE ANALYZER Model UV-100) and showed results in Fig. 4. After 60min discharges under various gas conditions, we obtained the ozone concentration of about 1ppm in the case of N_2, about 80 ppm in air, about 100 ppm in N_2/O_2 of 80/20 and about 460 ppm in N_2/O_2 of 50/50. It is well known that the ozone has strong oxidation power, and inactivation effect for fungi and bacteria. [9]-[10] With an increase of O_2 gas addition, the UV effect decreases and ozone increases as shown in Figs. 3 and 4. As shown in Fig. 2, it is seen that the D value in Fig. 2(a) is slightly shorter than that in Fig. 2(b). Therefore, we can speculate that the effect of UV emission is rather stronger than the effect of ozone addition in the case of N_2:O_2 of 80:20. When the O_2 gas mixture ratio increases from 20 % to 50 %, then it is clearly that the inactivation occurs more rapidly. By comparing survival curves shown in Figs. 2(b) and (c), it is found that surviving spores in Fig. 2(b) are less than that in Fig. 2(c) about two orders or more at the same treatment time from 10 min to 60 min. This might be due to the fact the denser ozone concentration was generated by DBD under more abundant oxygen gas. In the actual humid air, we can confirm more rapid sterilization, as shown in Fig. 2(d).

To investigate the spore shape after plasma irradiation for 60 min, we performed the morphology analysis of the spores by using the SEM. Figure 5 shows the spore shapes before and after plasma treatment.

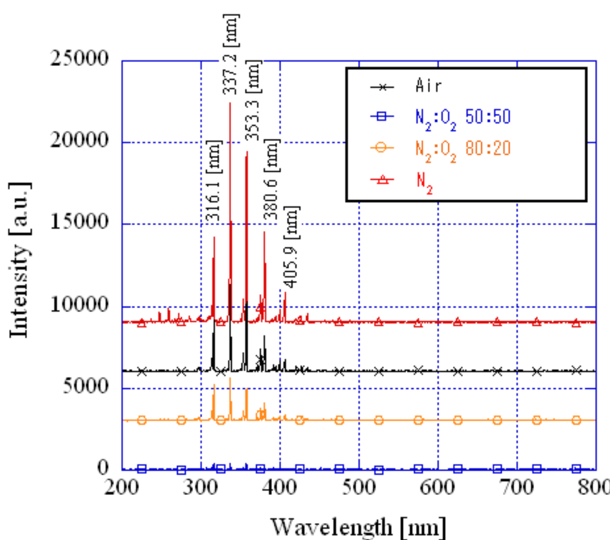


Fig.3 Emission spectra of DBD plasmas under various gas conditions of 100% N_2, N_2:O_2 of 80:20, N_2:O_2 of 50:50, and air under atmospheric pressure.

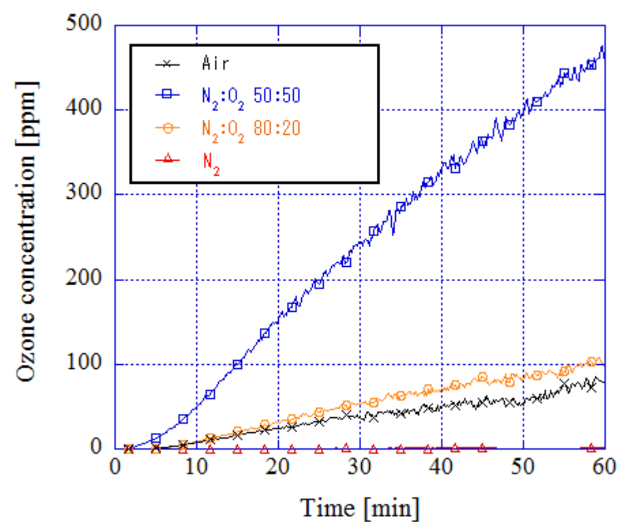


Fig.4 Ozone concentration in the chamber of during plasma discharge under various gas conditions of 100% N_2, N_2:O_2 of 80:20, N_2:O_2 of 50:50, and air under atmospheric pressure.

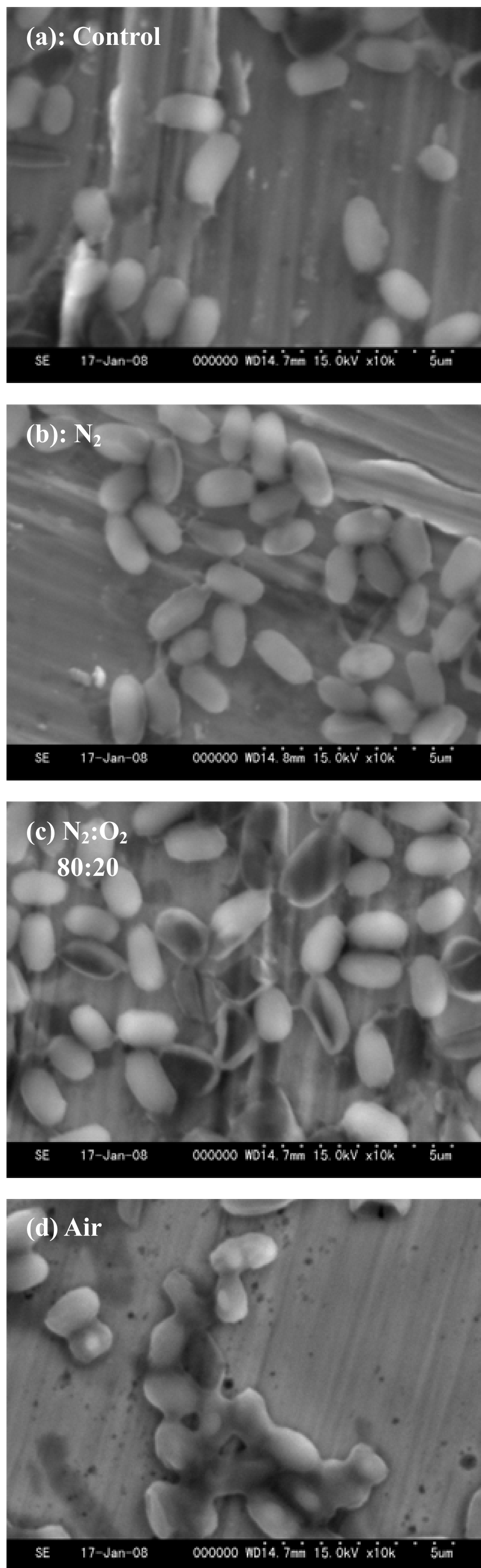
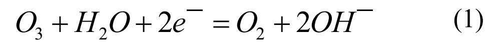


Fig.5 SEM images of *G. stearothermophilus* spores, (a) before plasma irradiation and after 60 minutes irradiation by line-shaped DBD plasma using (b) N_2, (c) mixed N_2: O_2 80:20 and (d) actual air (at 27ºC, 67%RH).

It is interesting to note that in the case of dry air, the spore shapes changed significantly to opened structure, and in the moist air, we confirmed that and in the moist air, we confirmed that bacteriolysis occurred. It is considered that it occurred via synergetic interaction between the ozone generated by DBD and water vapor in the air under the DBD plasmas, as shown in Eq. (1). The possibilities of OH radicals generated by direct discharging water molecules in the air and/or ultraviolet irradiation are also considered.



$$O_3 + H_2O + 2e^- = O_2 + 2OH^- \qquad (1)$$

Lastly, we will briefly mention about the atmospheric pressure twisted wire-type DBD plasma. Figure 6 shows photograph of twisted wire-type DBD electrodes set inside the resin tube. Two insulated copper wires with diameter of 0.2mm, were twisted spirally. Wire-type DBD plasma was stably generated, applying a voltage of ± 0.75kV at a frequency of 5 kHz between the two copper wires. Using thin wire-type DBD plasmas, the inner sterilization of the narrower tube will be demonstrated. These results will be given elsewhere.

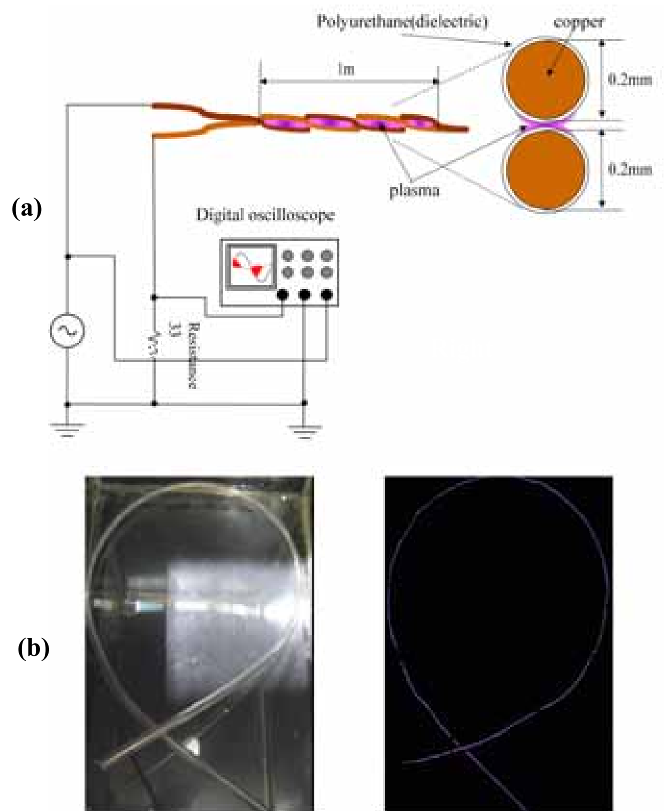


Fig. 6 (a)Schematic drawing of experimental setup of a twist wire-type electrode and (b)photographs of wire electrode inserted inside resin tube before (left) and after (right) DBD ignition.

4. Conclusion

We carried out sterilization experiments inside thin tube using a linear DBD under atmospheric pressure. We measured UV emission spectrum and ozone concentration generated by linear DBD under various gas conditions. We demonstrated the faster sterilization of

Geobacillus stearothermophilus having a population of 10^6 spores by 10min plasma irradiation in the case of moist air. We deduced that the sterilization factors are synergetic effects of UV emission, ozone and OH radicals, generated by discharge and/or UV excitation of water molecule in the air.

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