Effects of oxygen radicals in low-pressure surface-wave plasma on sterilization

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The effects of oxygen radicals on sterilization were studied using a 2.45 GHz surface-wave oxygen plasma. A population of 1.5×10^6 *Bacillus stearothermophilus* spores was irradiated for 3 min or more with oxygen plasma, generated at pressures between 6 and 14 Pa. The decimal reduction value (*D* value), a measure of the effectiveness of sterilization, was determined to be about 15–25 s. Using only oxygen radicals, excluding all charged particles, the 1.5×10^6 spores were sterilized with a *D* value of 30–45 s after 5 min or more of irradiation. On scanning electron microscopy, the length and width of the spores changed significantly due to chemical etching by oxygen radicals.
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The methods used in the sterilization of medical instruments depend on the heat resistance of the instruments. Traditionally, for heat resistant instruments, either dry heat or hot steam sterilization is used, while, for heat sensitive instruments, sterilization with ethylene oxide is used. The disadvantage of the dry heat or hot steam sterilization is that they can only be applied to instruments that can withstand the high temperatures. The disadvantages of ethylene oxide gas sterilization are that ethylene oxide is toxic and poses environmental risks. As well, it takes one or more weeks for the ethylene oxide levels to return to safe levels, which is much longer than the time required to sterilize the equipment. Another type of sterilization method, which combines hydrogen peroxide gas with an rf discharge plasma, is commercially available.¹ Currently, this equipment is assumed to effectively sterilize due to the high concentration of hydrogen peroxide rather than to the plasma component.

At present, various low-temperature plasma sterilization techniques have been developed, such as low-pressure glow discharges,² glow discharges at atmospheric pressure,^{3–5} downstream plasma produced by microwave excitation,^{6–8} moving atmospheric microwave plasmas,⁹ and surface-wave plasmas.^{10,11} The plasma sterilization method has certain advantages over conventional methods, including a low sterilization temperature (often less than 70 °C) and a short sterilization period of several minutes to 1 h.

Previously, we reported that a six-log reduction in spores could be achieved after only a 3 min irradiation with a low-pressure oxygen surface-wave plasma.¹¹ In this study, we studied the effect of oxygen radicals on sterilization.

The experimental setup used has been described by Nagatsu *et al.*¹¹ The 2.45 GHz microwave guided by a rectangular waveguide was fed into the stainless-steel chamber (diameter of 40 cm and height of 40 cm) filled with oxygen gas

through slot antennae cut in the broad face of each waveguide.¹² The launched microwave power varied from 0 to 1.5 kW. The vacuum chamber's pressure was lowered using a turbomolecular pump to a pressure of an order of 10^{-3} Pa. We compared the sterilizing characteristics of oxygen, hydrogen, and argon discharges at low pressures of about 6–14 Pa and a gas flow rate of 200 sccm. A population of 1.5×10^6 *Bacillus stearothermophilus* spores (ATCC No.12980) was placed on small stainless-steel disks and used as the biological indicator.¹³ The disks were placed in Petri dishes set onto a substrate stage with a diameter of 200 mm about 15 cm below the quartz vacuum window. A microwave having an incident power of 750 W was launched into the chamber in order to produce the plasma. The plasma irradiation period varied from 0 to 10 min. The stage temperature was measured using a thermocouple attached below the metal stage and thermolabel sheets attached on the rear surface of the Petri dish. After plasma irradiation, the spores were incubated in culture tubes for 7 days at an incubation temperature of 55–60 °C as is standard for *Bacillus stearothermophilus*.

Figure 1 shows the colony count results of the *Bacillus stearothermophilus* spores irradiated with oxygen plasma. As shown in Fig. 1, the plotted data roughly lie on a straight line. From the slope of the line, we calculated the decimal reduction values (*D* value) to be approximately 15–25 s. When the plasma irradiation lasted for more than 3 min, it was found that the number of colony forming units was zero. In Fig. 1, we also plotted the inverse of the results of the experiments that yielded a zero spore count at 2.5 min (0.5) and at 3 min (0.1). Furthermore, to compare the effect of gas species on sterilization, we tested argon and hydrogen plasmas using the same discharge condition: A microwave power of 750 W, a pressure of about 10 Pa, and irradiation duration of 10 min. However, as shown in Fig. 1, for both the argon and hydrogen plasma discharges, the spores survived even after a 10 min irradiation. From this, we can deduce that

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FIG. 1. Survival curves of the *B. stearothermophilus* spores irradiated by the oxygen, hydrogen, and argon surface-wave plasmas.

electrons and ions are not the primary agents in this sterilization process. Consequently, it might be the oxygen plasma that plays a role in killing the spores in this experiment. It should be noted that the stage temperature, based on the thermocouple attached below the metal stage and the thermolabel sheets attached on the rear surface of the Petri dish, was always less than 70 °C for all of the different gas species. Figure 2 shows the change in the stage temperature as measured using the thermocouple versus the plasma irradiation periods for the different gas species of Ar, H₂, and O₂. After a 10 min plasma discharge, the temperature increased by approximately 25 °C in all cases. Based on the thermolabel measurements, it was also shown that the surface temperature of the Petri dish was less than 55 °C after a 10 min plasma discharge.

To study the shape and size of the spores before and after plasma irradiation, we carried out measurements using a scanning electron microscope (SEM). It was shown that the length and width of the spores dramatically decreased after only a 4 min plasma irradiation. The ellipsoidal spores with a length of 1.5 μm and a width of 0.9 μm shrank to jellybean-like slender shapes.¹¹ This could be attributed to chemical etching by the oxygen radicals generated in the oxygen plasma. However, in the case of the argon or hydrogen plasma, it was found that the sizes and shapes of the spores were not affected by a 10 min plasma irradiation.

In order to examine the effect of the UV radiation emitted from the oxygen plasma, we placed a thin quartz plate with a thickness of 3 mm over the Petri dish to block any

FIG. 2. The change in the stage temperature measured with the thermocouple vs the plasma irradiation periods for the different gas species: oxygen, hydrogen, and argon.

FIG. 3. Survival curves of the *B. stearothermophilus* spores irradiated with oxygen radicals and nitrogen surface-wave plasmas.

charged or neutral particles, while only UV photons with a wavelength, λ , greater than 160 nm were able to penetrate the quartz plate. However, after a 10 min oxygen plasma discharge, the spores were still alive and had not changed their size or shape. From these results, it would appear that UV radiation is not necessary for this sterilization to be effective in the present experiment. However, it could be the case that, as the radicals approached the Petri dish, UV photons might be generated. To further investigate the role of UV photons, future experiments using optical emission spectroscopy need to be performed.

To examine the effect of oxygen radicals on sterilization, we placed a stainless-steel plate with 4 mm diameter holes on a stage located 8 cm below the quartz window. Although the electrons and ions were blocked by this punched plate, the neutral radicals could pass through the plate, together with any UV photons that might have a weak effect on sterilization. We measured the survival curve of the *Bacillus stearothermophilus* spores in a Petri dish located 7.5 cm below the punched plate. In Fig. 3, the number of colony form-

(a)

(b)

FIG. 4. SEM images of *B. stearothermophilus* spores (a) before and (b) after a 10 min oxygen radical irradiation.

ing units for different plasma discharge periods is shown. From the slope of the survival curve, we found the D value to be roughly 30–45 s. The SEM images of the original spores are shown in Fig. 4(a), while the images after a 10 min radical irradiation are shown in Fig. 4(b). As in previous experiments, it can be seen that the spores changed their shape and size.¹¹ Hence, we concluded that the primary sterilization species were the oxygen radicals produced by the surface-wave sustained oxygen plasma.

Recently, we performed an analogous experiment using nitrogen plasma discharge in order to study its effect on sterilization. From a colony count, we found that the spores were sterilized after a 4 min discharge. A preliminary result of the colony count is also plotted in Fig. 3, where the D value was roughly estimated as 30–40 s, although there is insufficient data for conclusive results. Furthermore, it is interesting to note that the spore shapes were almost identical to the original ones, which differs from the findings with oxygen plasma or oxygen radical irradiation. This result could possibly be caused by UV photons emitted by the nitrogen plasma. Similar experiments have been done by Moisan's group in the microwave plasma with N_2 – O_2 gas mixtures and they showed that UV emission ranging from 250 to 320 nm was strongly enhanced by adding 2% of O_2 gas content into N_2 plasma.^{7,8} To confirm the role of UV photons emitted directly from the plasma or those generated when the radicals approach the Petri dish, an examination using UV emission spectroscopy is planned, and we hope to report the results in the near future.

In conclusion, we studied the effect of oxygen radicals produced in a surface-wave plasma discharge on sterilization. Experimental results showed that the *Bacillus stearothermophilus* spores were sterilized at a low temperature by oxygen plasma irradiation after 3 min or more or by oxygen radical irradiation after 5 min or more. We also tested argon and hydrogen plasma discharges using the same discharge conditions and found that sterilization was not achieved even after a 10 min plasma discharge. From these empirical re-

sults, it might be concluded that the sterilization mechanism in the oxygen plasma is due to a chemical etching reaction from the reactive oxygen radicals. Further studies will be required to examine the role of UV emission in nitrogen plasma discharges.

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