Generation Technique and Sterilization Application of Microwave-excited Plasma inside a Medical Container

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A novel technique of generating stable microwave-excited plasma inside a medical container is proposed. With this technique, sterilization process can be easily carried out with the medical instruments stored in the medical container without opening it just like the sterilization process what is carried out by autoclave. Biological indicator (BI) with 2.3×10^6 *Geobacillus stearothermophilus* spores are placed at the center bottom of the medical container to check the sterilizing effectiveness of the plasma excited by 2.45 GHz microwave launched from the plastic cover of the medical container. By using air plasma with gas flow rate of 50 sccm, when net microwave power is kept 400 W at pressure of 25 Pa, the BI samples are sterilized after 15 min plasma irradiation in the preliminary experiments. In the latest experiments, sterilization of the same BI samples can be realized after 15 min plasma irradiation at air flow rate of 200 sccm, when net microwave power is kept 300 W at pressure of 95 Pa.

Keywords: microwave plasma, plasma sterilization, internal sterilization, medical container, air plasma.

1. Introduction

Conventional methods of sterilizing medical instruments have been mainly performed using a dry-heat or hot-steam autoclave for heat-resistant materials, and ethylene-oxide gas for heat-sensitive materials. However, there are several drawbacks of the conventional methods of sterilization and disinfection. For example, the former method is limited to use the treated materials, such as the metal or glass implements. As for the ethylene-oxide gas (EOG) sterilization, the environmental problems due to its toxicity are recently concerned. Therefore, it is desired to develop a new type of sterilization technique, which makes it possible to sterilize the medical instruments safely and rapidly at low temperature.

Up to now, various low-temperature plasma sterilization techniques have been developed using low-pressure glow discharges [1], glow discharges at atmospheric pressure [2-4], down-stream plasma produced by microwave excitation [5], moving atmospheric microwave plasma [6] and surface-wave plasma [7-10]. The plasma sterilization method has a number of advantages compared with the conventional methods, such as sterilization is able to be performed at a relatively low temperature and in a relatively short period.

So far, the plasma sterilization technique has been studied mainly in surface sterilization of medical instruments. In order to sterilize the medical instruments stored inside the medical container without opening it just like the sterilization process what is carried out by autoclave, recently our group has proposed a novel technique of generating stable microwave-excited plasma inside a medical container (square cross section = 27×27 cm^2, height = 15 cm, metal box with plastic cover) for sterilization application to solve the problem mentioned above.

2. Experimental setup

Schematic drawing of an existing 60-cm-diameter microwave plasma device used in preliminary experiments is shown in Fig. 1. A 2.45 GHz microwave, guided by a rectangular waveguide and coaxial waveguide, is fed into a vacuum chamber filled with discharge gas through microwave launcher. The cylindrical vacuum chamber has 60 cm in diameter and 35 cm in height, and can be evacuated to the order of 1 Pa by a rotary pump. The microwave power can be varied from 0 to 1.5 kW.

Proto type of a compact microwave plasma device designed for plasma sterilization inside medical container has the same structure of coaxial waveguide and microwave launcher as the 60-cm-diameter device. The vacuum chamber has 75 cm in length, 42 cm in width and 32.5 cm in height, and can be evacuated to the order of 10^{-3} Pa with a turbo-molecular pump. In addition, it has a horizontal slide stage, whose perpendicular position can be adjusted outside the vacuum chamber. The microwave power can be varied from 0.2 to 3 kW.

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The plastic cover of medical container can act as dielectric for transferring the 2.45 GHz microwave to generate plasma inside container. Perpendicularly movable stage can lift up medical container to approach microwave launcher. Dielectric materials such as glass fiber and silicon sheet are used as cushion to fill the gap between microwave launcher and container. A viewing port is set on the side wall of container for observing plasma generation. In plasma sterilization experiments, BI samples are placed at the center bottom of the medical container to check the sterilizing effectiveness of the plasma excited by 2.45 GHz microwave launched from the plastic cover of the medical container. During plasma irradiation, thermal-label sheets are attached to the Petri dish placing with BI samples and the downside of container cover to measure the temperature.

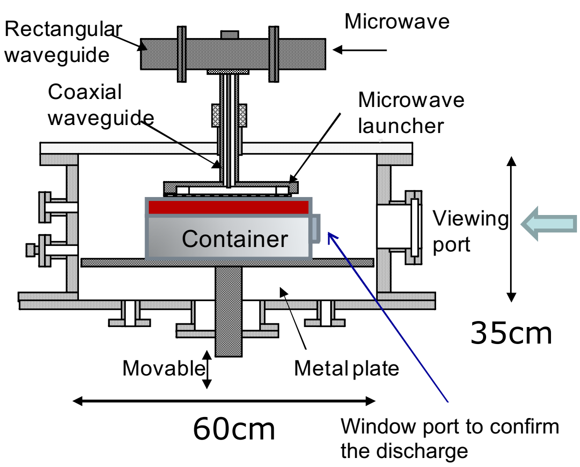

Fig. 1 Schematic of 60-cm-diameter microwave plasma devices.

The BIs used have an oblong polished stainless steel disc carrier for two species of non-pathogenic spore-forming bacteria: *Geobacillus stearothermophilus* spores with a population of 1.9~2.3×10^6; *Bacillus atrophaeus* (former *Bacillus subtilis* variety niger) spores with a population of 4.0×10^6. After plasma irradiation, the spores were incubated in prepared culture solution for seven days or more at an incubation temperature of 55~60°C, as is standard for *G. stearothermophilus*, and at an incubation temperature of 30~35°C, as is standard for *B. atrophaeus*. Subsequently, it can be determined whether the spores have been killed or not because the colour of the culture solution change from purple to yellow if the spores are still viable. For the colony count by the spread plate method, each of BI samples is put in a 1.5 ml BHI solution separately to obtain the original solution. Then, a 0.1 ml pick-up from the original solution is spread uniformly on the surface of a LB agar plate. Having incubated inverted at standard incubation temperature, the number of colonies formed by viable bacteria is counted.

The morphology of the spores has been analyzed with SEM measurement.

3. Results and discussion

3.1 Preliminary experiments

An existing 60-cm-diameter microwave plasma device in our laboratory was used to study optimal discharge conditions for generating microwave-excited plasma inside the medical container. A piece of glass fiber is used as cushion to fill the gap between launcher and container. Whether plasma is generated or not inside the medical container can be checked by the viewing port on the side wall of the container via the viewing port of the 60-cm-diameter chamber. When the microwave power was 400 W at pressure of 25 Pa with 50 sccm air flow, stable air plasma can be generated as shown in Fig. 2.

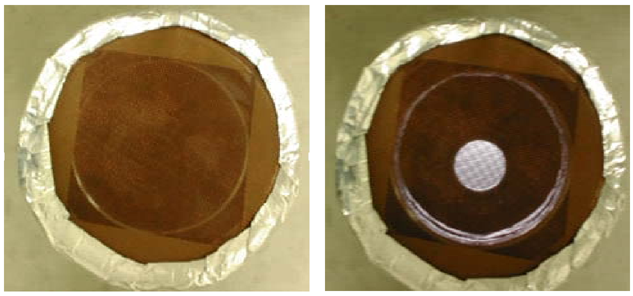

Fig. 2 Photograph of microwave-excited air plasma generated inside the medical container.

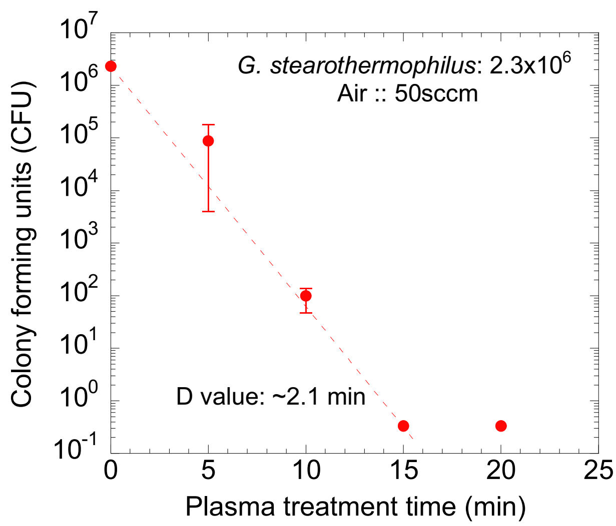

Fig. 3 Survival curves of *G. stearothermophilus* spores treated by air plasma generated inside the medical container.

In the plasma sterilization experiments, BI samples with 2.3×10^6 *G. stearothermophilus* spores are placed at the center bottom of the medical container. To study the survival curves of spores with the agar colony count method, the net plasma treatment time was varied from 0 to 20 min by 5 min step. Figure 3 shows the survival curves in case of 400 W air plasma sterilization. It was found that the 2.3×10^6 spores were killed after about 15 min of air plasma discharge with roughly linear slope, and the D-value was evaluated to be about 2.1 min. Time-modulated discharges were applied as on/off-time = 30/120 s to avoid heat damage to the plastic container cover. After 20 min net plasma treatment, thermal label sheets showed the temperature was around 90-95 °C for the BI samples and was around 120-125 °C for the downside of container cover.

In the case of BI samples with $4.0\text{x}10^6$ *B. atrophaeus* spores placed at the center bottom of the medical container, the net plasma treatment time was varied from 0 to 15 min. Figure 4 shows the survival curves in case of 380 W air plasma sterilization. It was found that the 4.0×10^6 spores were killed after about 15 min of air plasma discharge with roughly linear slope, and the D-value was evaluated to be about 1.8 min. Time-modulated discharges were also applied as on/off-time = 30/120 s to avoid heat damage to the plastic container cover. After 15 min net plasma treatment, thermal label sheets showed the temperature was around 80-85 °C for the BI samples and was around 95-100 °C for the downside of container cover.

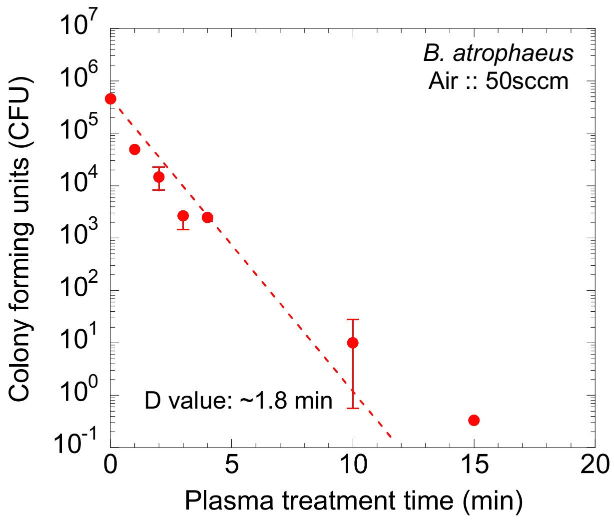

Fig. 4 Survival curves of *B. atrophaeus* spores treated by air plasma generated inside the medical container.

SEM measurement had been carried out to study spore morphology. By comparing the SEM images of BI samples sterilized by air plasma inside container and the non-plasma treated control, significant reduction of spore sizes was found as shown in Fig. 5. We considered the spores were etched by oxygen radicals originated from oxygen plasma [8, 9].

This result indicated that BI samples were exposed to direct air plasma irradiation inside the medical container. That means it is possible to realize this kind of microwave-excited plasma generation technique inside the medical container and it is an ideal method to sterilize the medical instrument stored in the medical container.

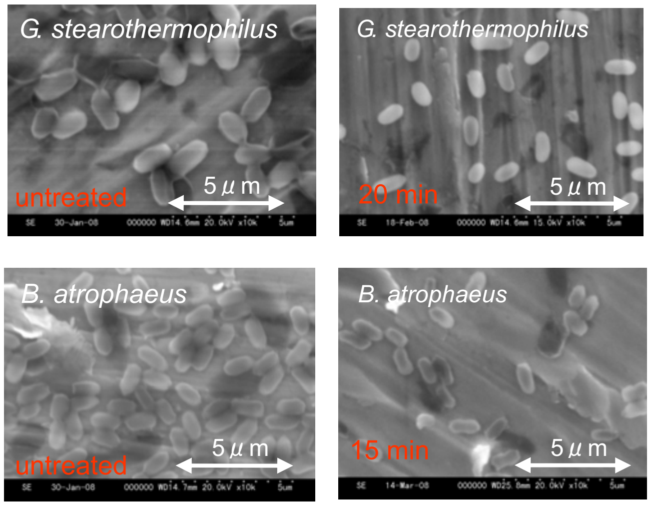

Fig. 5 Comparison of SEM images of spores.

3.2 Proto type for plasma sterilization inside container

Since the preliminary experiments carried out with 60-cm-diameter microwave plasma device proved that coaxial waveguide and microwave launcher structure was suitable for generate of microwave-excited plasma inside the medical container, a proto type of compact microwave plasma device has been designed for plasma sterilization inside medical container with the same structure.

First, sterilization experiments of BI samples placed directly under the microwave launcher were carried out. When the net microwave power was 400 W at pressure of 64 Pa with 25 sccm air flow, stable surface-wave plasma was generated near the microwave launcher as shown in Fig. 6(a). If we kept the distance between movable stage and the microwave launcher at minimal value of 12 cm, BI samples with 1.9×10^6 *G. stearothermophilus* spores placed under the center of microwave launcher were sterilized by 15 min direct air plasma irradiation at temperature lower than 50 °C. When the net microwave power was 400 W at pressure of 7 Pa with 100 sccm air flow, diffused plasma was generated to the downstream region as shown in Fig. 6(b). The BI samples placed under the center of microwave launcher were sterilized by only 4 min direct air plasma irradiation at temperature of about 60 °C. Moreover, water vapor addition could reduce the sterilization time to 3 min. It seemed that the air plasma generated at pressure of several Pa order diffused well and had better sterilizing effectiveness.

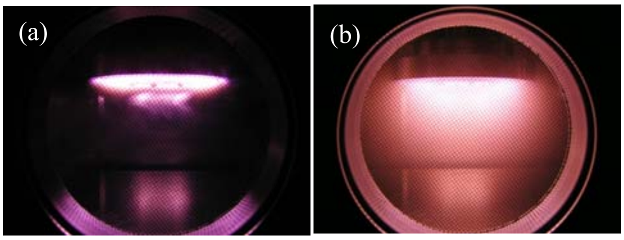

Fig. 6 Photographs of air plasma generated at different pressure of (a) 64 Pa with 25 sccm air flow, (b) 7 Pa with 100 sccm air flow.

Next, the medical container was placed under the microwave launcher. The medical container was lifted up by perpendicularly movable stage to approach launcher and a piece of silicon sheet was used as cushion. Pressure was adjusted to find the optimal value to generate plasma only inside the medical container with different gas species such as helium, nitrogen, oxygen and air. We could conclude that pressure at several tens Pa order is relatively easier for plasma generation inside the medical container.

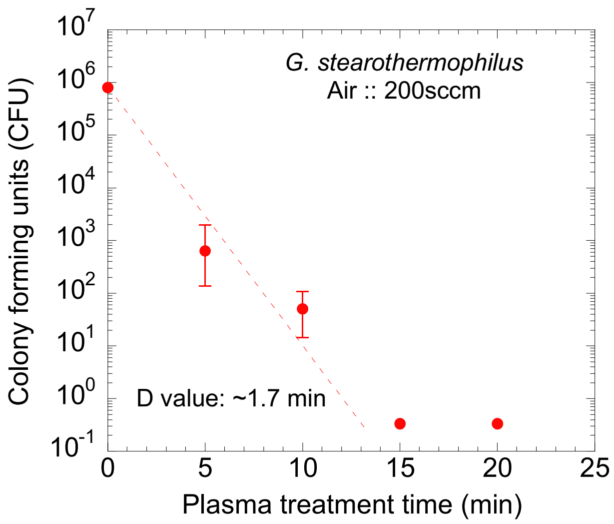

Fig. 7 Survival curves of *G. stearothermophilus* spores treated by air plasma generated inside the medical container.

In the plasma sterilization experiments inside container, BI samples with 2.3×10^6 *G. stearothermophilus* spores are placed at the center bottom of the medical container. The BI samples were sterilized by 15 min air plasma irradiation excited with 300 W microwave. Figure 7 shows the survival curves in case of air plasma sterilization at pressure of 95 Pa with roughly linear slope, and the D-value was evaluated to be about 1.7 min. Time-modulated discharges were applied as on/off-time = 30/90 s to prevent from high temperature. After 20 min net plasma treatment time, thermal label sheets showed the temperature was around 50 °C for the BI samples and was lower than 50 °C for the downside of container cover.

We will present the experimental results in detail in the poster session (FH1.P1-123).

4. Conclusions

In this study, a novel technique of generating stable microwave-excited plasma inside a medical container is proposed. With this technique, sterilization process can be easily carried out with the medical instruments stored in the medical container without opening it just like the sterilization process what is carried out by autoclave.

In the preliminary experiments, plasma generation inside a closed medical container was realized, and the BI samples with 2.3×10^6 *G. stearothermophilus* spores placed at the center bottom of the medical container were sterilized by 15 min plasma irradiation with 50 sccm air flow, when net microwave power was kept 400 W at pressure of 25 Pa. In the latest experiments using proto type for plasma sterilization inside container, the same BI samples could be sterilized by 15 min plasma irradiation with 200 sccm air flow, when net microwave power was kept 300 W at pressure of 95 Pa.

Plasma sterilization experiments inside the medical container with water vapor addition and sterilization of *B. atrophaeus* using proto type are also planned to be carried out in the near future.

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