22P004-O

Sterilization Treatment by Using Atmospheric Pressure Plasma Jet
大気圧プラズマジェットによる殺菌・滅菌処理

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Germicidal treatments of *Escherichia coli* (*E. coli*) have been performed using an atmospheric pressure Ar plasma jet sources. While the bactericidal effect confirmed under plasma treatment, the surface temperature of Luria-Bertani (LB) agar was not increased and ultraviolet (UV) is low emission. Therefore, it is considered that ions and radical species generated by atmospheric pressure plasma jet have bactericidal effects.

1. Introduction

Sterilization treatment is of importance to prevent the infection disease and food poisoning. Today, it is necessary to select the sterilization method according to microbial species or the character of sterilizing objects. Therefore, research of the new sterilization method is attractive. The plasma sterilization also start to garner attention as a new sterilization method.[1,2)] In addition, atmospheric pressure plasma is studied for various uses these days.[3,4)]

We have evaluated the bactericidal effect of the plasma using *E. coli* as a biological indicator the index in UV, radical species and heat by use of atmospheric pressure plasma jet.

2. Experimental Methods

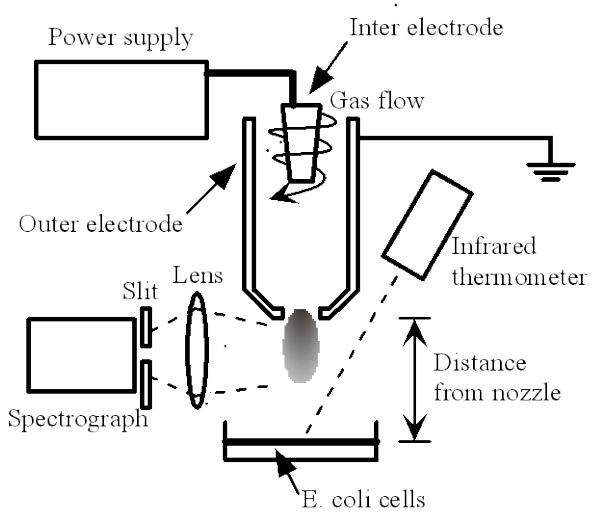

Fig.1. Schematic of the germicidal treatment by plasma jet, spectroscopic measurement, and thermometry.

A schematic of our experimental set-up is shown in Fig.1. The plasma device is an atmospheric pressure plasma jet source (Plasma-Pre-treatment System; Plasmatreat), which consists of a conical inner electrode, and a grounded outer electrode with a nozzle of 5 mm diameter. The inner electrode is coupled to stepped high frequency pulse current power supply, about 280 V, 8 A, and 16-20 kHz, through a high voltage transformer (HTR1001; Plasmatreat). The working gas is Ar, and flows spirally at 20-50 l/min through two electrodes.

The spectroscopic diagnostic system used consists of a space-resolving slit of 1 mm width, a collecting lens, and spectrograph (Maya2000PRO; ocean optics). The spectrograph allows measurement wavelength between 200 and 1000 nm. The schematic layout of the spectroscopic diagnostic system is also shown in Fig.1.

In this research, *E. coli* was employed as the indicator bacterium in order to evaluate the bactericidal effect of the plasma. Indicator samples were prepared by the following procedures. In the first place, 10ml LB agar were poured into glass laboratory dishes (inside diameter: 40 mm, height: 20 mm). Then, 100 μl suspensions of overnight grown *E. coli* culture (10^8 colony forming unit (CFU)/ml) were evenly spread over LB agar in the dishes. The dishes were exposed to the plasma with increasing time. Temperature of the surface of the agar was monitored during the exposure using the infrared thermometer (THERMO-HUNTER; OPTEX). After the exposure, the exposed portion of the LB agar was excavated from the dish and cells were recovered and diluted serially with sterilized liquid. One hundred microliter of each

dilute was spread in LB agar plates and incubated for 24 hours at 37 °C.[5)]

3. Results and Discussion.

UV and visible emission spectra are shown in Fig.2 in the wavelength range from 200 to 1000 nm, where the observation positions are 5, 10 and 15 mm from nozzle, and the working gas was Ar. The strong emission line of the Ar I was observed in the wavelength range from 650 to 900 nm. Moreover, the OH A-X transition lines were found around 310 nm, therefore radical species were generated from not only Ar gas but atmosphere. In other words, reactive oxygen species (•OH, $•O_2$ and etc) may be generated from H_2O contained in the surrounding atmosphere by plasma exposure. When the observation position was near from nozzle, the intensity of the emission spectra was strong.

Fig.3 shows that the total counts of the surviving bacteria on the LB agar after plasma treatment (the working gas was Ar gas, the gas flow rate was 20 l/min, the plasma exposure time was 60 seconds, the distance from nozzle was 5 mm, 10 mm, and 15 mm). As a result, significant bactericidal effect was seen on *E. coli* by the plasma treatment. Furthermore, when the distance between the nozzle and LB agar was small, the bactericidal effect was higher. However, as shown in Fig.2. emission intensity of UV (200-300 nm) was low. Moreover, measured temperature on the surface of the LB agar under each plasma treatment was less than 40 °C.

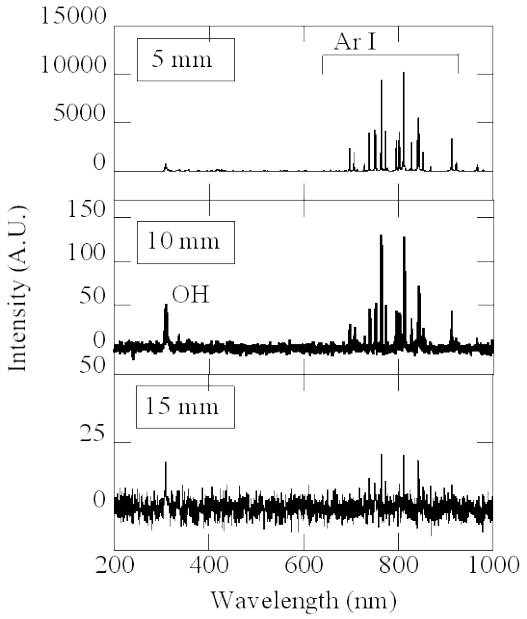

Fig. 2 UV/visible emission spectra of Ar plasma jet sources in the wavelength range from 250 to 1000 nm, where distance from nozzle

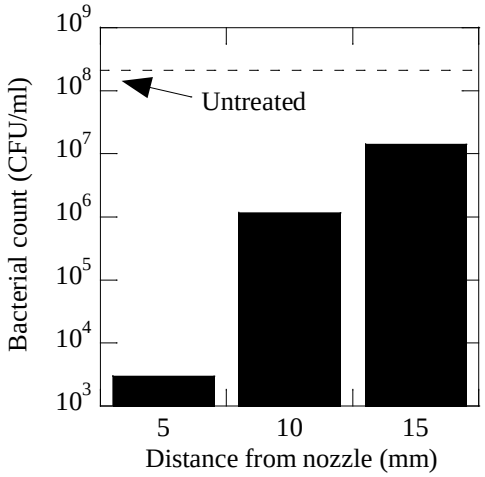

Fig.3. Total bacterial count of Escherichia coli after plasma treatment.

For this reason, it is considered that this bactericidal effect was not high within the range of UV emission and heat in this expriment.[6,7)] As we discussed in the results, the bactericidal effects on E. coli would be caused by ions and radical species produced in the plasma.

4. Conclusion

In this study, we have investigated the bactericidal effects on *E. coli* using atmospheric pressure plasma generated from Ar gas. The bactericidal effects caused by the plasma would be other than UV or heat from the plasma. When the distance from nozzle was small, ions or radical species increase and the bactericidal effect was higher. It is considered that bactericidal effects in ions and radical species generated by atmospheric pressure plasma jet might be more significant.

References

[1] Lassen, K. S., Nordby, B., and Grun, R: J. Biomed Mater Res Part B, Appl Biomater, **65B** (2003) 239.

[2] S. Takashima, and M. Kamitani: Japanese Society of Medical Instrumentation, **78** (2008) 49.

[3] Y. Takemura, N. Yamaguchi, and T. Hara: J. Appl. Phys. **47** (2008) 5644.

[4] Y. Takemura, Y. Kubota, N. Yamaguchi, and T. Hara: Ieee Transactions on Plasma Science, **37** (2009) 1604.

[5] R. F. Harris, and L. E. Sommers: American Society for Microbiology, **16** (1968) 330.

[6] S. Kinoshita, and K. Yoshino: Society for Antibacterial and Antifungal Agents, Japan, **38** (2010) 521.

[7] T. Tatsuta, and T. Ogura: Biophysical Society of Japan, **39** (1999) 295