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Investigation of Sterilization Effect for Overlapping Pieces in Non-Thermal Sterilization Method of Packaged Fresh Foods Using Pulsed Barrier Discharge

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Abstract: It is important to secure food safety. If a packaged food can be sterilized, food poisoning can be reduced considerably because the packaged food in a plastic container is useful to prevent attaching microorganisms. However, a fresh food, e.g., a salad or sashimi, cannot be sterilized by thermal sterilization. Therefore, we are studying sterilization methods for packaged fresh foods, such as packed salads. In this study, we have investigated the applicable probability of the sterilization method using cold plasma applied to the packaged fresh food. Especially, we have investigated the probability of the sterilization of microorganisms living in a small hollow between the overlapped fresh foods. A plastic petri dish with a lid was used for simulating the plastic container. The cold plasma was applied into the petri dish by a barrier discharge generated by a pulsed voltage. *E. coli* was used for a target of sterilization. The *E. coli* was set on a culture agar medium instead of the salad. The experimental results showed that the sterilization method combining the plastic film and cold plasma of the barrier discharge generated by the pulsed voltage is applicable to sterilize microorganisms living in the hollow between the overlapped fresh foods for the packaged fresh food.

Keywords: packaged fresh foods; salad; overlapped fresh foods; hollow; barrier discharge; sterilization; non-heating

Citation: Saito, K.; Onuma, T.; Ota, D.; Sato, Y.; Minamitani, Y. Investigation of Sterilization Effect for Overlapping Pieces in Non-Thermal Sterilization Method of Packaged Fresh Foods Using Pulsed Barrier Discharge. *Agronomy* **2022**, *12*, 822. https://doi.org/10.3390/agronomy12040822

Academic Editor: Milena Petriccione

Received: 29 November 2021
Accepted: 23 March 2022
Published: 28 March 2022

1. Introduction

It is important to secure food safety for living. However, incidents that threaten the safety of the food have occurred frequently. Every year, many people suffer from food poisoning by eating a food sterilized insufficiently. Sterilization of the foods is indispensable when the safety of the foods is considered. For prevention of food poisoning, sufficiently sterilizing the foods and preventing microorganisms attaching to the foods are necessary. Meanwhile, packing the food in a bag is useful to prevent attaching microorganisms. If the packaged food can be sterilized, the incidence of food poisoning can be reduced considerably.

Currently, foods are mainly sterilized by using heat, chemicals, and ultraviolet rays for the purposes of the prevention of food poisoning and long-term preservation of the foods. However, these sterilization methods have merits and demerits. The heat can sterilize the food inside the bag. However, fresh food, e.g., a salad, cannot be sterilized by the heat. The chemicals cannot sterilize the food inside the bag. Moreover, the ultraviolet rays have difficulty to sterilize the food inside the bag because the ultraviolet rays cannot pass through most of the materials of the bags. Currently, radiation sterilization is the only method that can sterilize packaged fresh foods, but radiation sterilization is not approved in Japan for safety reasons. Therefore, we are studying sterilization methods for solving these problems for packaged fresh food.

Some studies of the sterilization of packaged fresh food have been carried out. Klockow studied the sterilization of a leaf of spinach by packing ozone along with it into a bag [1]. Misra studied the inactivation of a surface of strawberry packaged in a bag by cold plasma generated by barrier discharge [2].

The cold plasma is attractive as the non-thermal sterilization method for fresh food. Since the cold plasma has a non-thermal equilibrium state where only electrons have high energy, it hardly heats the food if it is used for sterilization. The cold plasma can also be generated in the atmosphere without lowering the pressure by applying a pulsed voltage. The cold plasma sterilization is a chemical and physical method [2–16]. The cold plasma generates ultraviolet rays and active species, such as ozone and OH radical. Active species exert an oxidizing effect on the cell membrane of a microorganism and destroy it [6–8,15,16]. Ultraviolet rays attack DNA through the cell membrane and stop the function of the DNA. By way of them, the cold plasma sterilization has a strong sterilizing effect [3,7–9,14].

In addition, the cold plasma is able to be generated in the space that is enclosed by dielectric materials and where electrodes do not touch directly [2,3,5–9,14–17]. Its method is called barrier discharge. Therefore, the plasma can be generated on the inside of the package by using the barrier discharge. In fact, it has been studied as the sterilization method for packaged fresh food [2,14]. Therefore, we are also investigating the applicable probability of the sterilization method using the cold plasma generated by the dielectric barrier discharge using pulsed voltage to such packaged fresh foods as salad or sashimi packed into a container or a bag, as shown in Figure 1.

(**a**) (**b**)

(**c**)

Figure 1. Examples for packages of a salad (**a**,**b**) and sashimi (**c**).

In the bags and containers of the packaged salad and packaged sashimi, many pieces are packed, as shown in Figure 1. Therefore, the pieces are overlapped with each other. However, the effect of the plasma to the microorganisms between the overlapped pieces of the foods have not been considered yet. In this research, our goal is to develop a method to sterilize between the overlapped pieces of fresh foods, such as sashimi and salad, by the plasma. Therefore, in this paper, as basic research for developing the sterilization method between the overlapped pieces of the fresh foods, we have investigated the bactericidal situation of the microorganisms living at various circumstances in between the overlapped pieces by the plasma using a simulated food.

2. Experimental Setup

In this paper, as first step, we used a petri dish closed by a lid (OD φ 92 × T 18 mm, lid thickness 0.8 mm, dish thickness 0.8 mm) for simulating the container-shaped package, such as in Figure 1a,c. In this experiment, the petri dish material that was used is plastic (polystyrene, relative permittivity 2.4–2.6) because many packaging materials for the foods are plastic, such as polystyrene, polypropylene (relative permittivity 2.2–2.6), and polyethylene terephthalate (relative permittivity 2.9–3.0). Since polypropylene and polyethylene terephthalate have close relative permittivity to polystyrene, electrical effects are not so different. However, the thickness of the petri dish is different from that of the plastic containers, such as in Figure 1. For example, the thickness of the container of Figure 1a is 0.3 mm, and the thickness of the container of Figure 1c is 0.4 mm. Therefore, since there is probability that necessary applied voltage value for the sterilization is different, the investigation of this point is planned to be reported later.

In this experiment, an agar medium was used for simulating the foods. Conductivity of the agar medium is 10 mS/cm. This value is same as that of general foods. Escherichia coli (ATCC11229) was used as the sterilizing target microorganism. In the uncooked food, such as meat, fish, and salad, detection of *E. coli* is used for test of food contamination. If *E. coli* is detected from a sterilized food, it is suggested that it has had not enough sterilization.

Sample suspension including *E. coli* was obtained by culturing *E. coli* for 24 h within an incubator kept at 37 °C using Nutrient Broth (Oxoid Limited). The number of *E. coli* in the sample suspension was about 10^9 CFU/mL. The suspension with cultured *E. coli* was diluted 10^6 times using purified water and applied at about 10^3 CFU/mL to the agar medium in the petri dish. Then, the petri dish was cooled to 20 °C of nearly room temperature. We measured the number of *E. coli* after applying the pulsed plasma in the petri dish. For the measurement of the number of *E. coli*, a colony counting method was used.

Figure 2 shows a circuit for generating barrier discharge for applying the plasma. The circuit is composed of a capacitor, a pulse transformer, and a trigger gap switch. This circuit is operated by that a high voltage trigger pulse is inputted to the trigger gap switch from a trigger circuit; after that, the capacitor is charged by an HV charger. Inputting the high voltage trigger pulse, the gap switch is made conducting and the voltage of the capacitor is applied to the pulse transformer. Winding ratio of the pulse transformer is 1 to 6. The voltage is made stepping up by the pulse transformer and applied to electrodes that sandwich the petri dish. A repetition rate is controlled by an interval of signals outputted repeatedly from the trigger circuit.

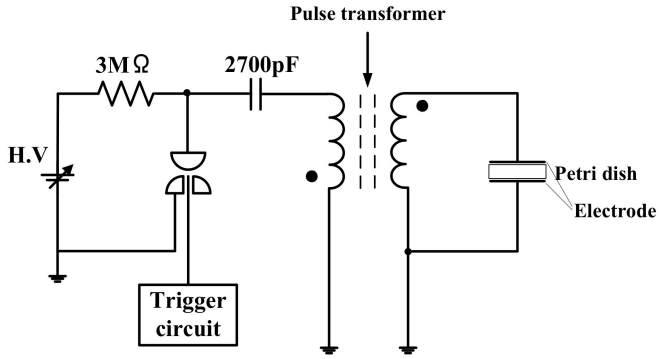
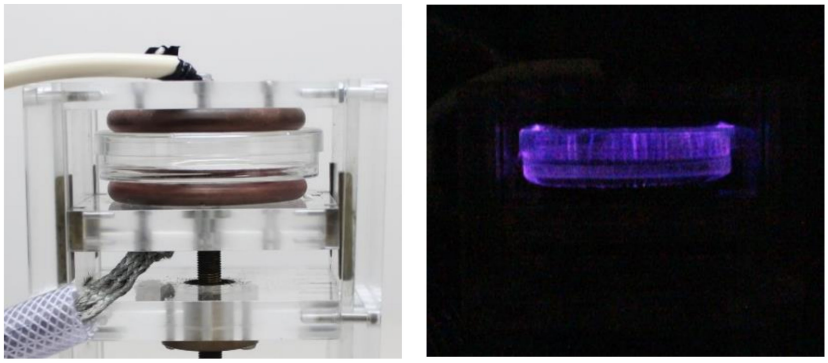


Figure 2. Pulsed plasma generating circuit.

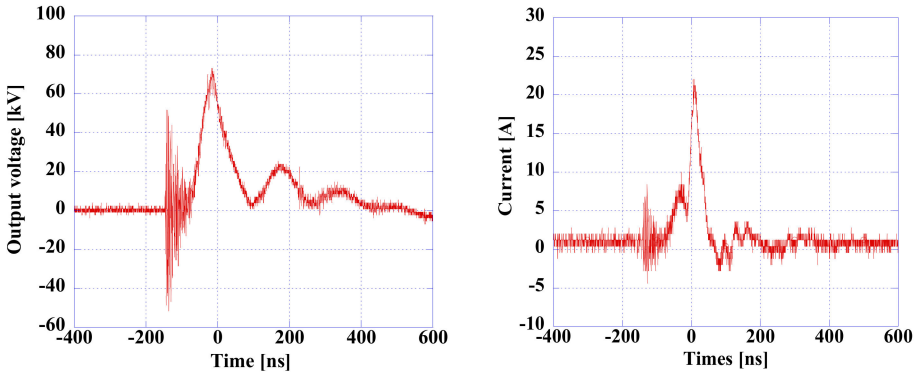
The pulsed plasma was generated in the petri dish for simulating the packaged food by applying a pulsed voltage. Pulsed voltage was applied to the petri dish put between the electrodes shown in Figure 3a. By that, the plasma occurred as in Figure 3b. Since the pulse voltage that was applied at 10 pps of the repetition rate and exposure time on a camera

was 1/4 s, the pulsed plasma was taken 2 times in picture of Figure 3b. The diameter and the thickness of the electrodes were φ 80 mm and 10 mm, respectively. Figure 4 shows the applied pulse waveforms at 10.5 kV of the charging voltage to the capacitor. The voltage waveform was measured by a self-making resistive voltage divider (7700:1). The resistive voltage divider was calibrated by comparing with a high voltage probe (Tektronix P6015A). The current waveform was measured by a current monitor (Pearson 6600, 0.1 V/A). Then, the pulsed voltage is 70 kV with 100 ns of the pulse width of FWHM.



(**a**) (**b**)

Figure 3. The electrode for applying pluses; (**a**) appearance of the electrode; (**b**) appearance of the generated plasma.



(**a**) (**b**)

Figure 4. Typical applied waveforms to the petri dish (charging voltage: 10.5 kV); (**a**) voltage waveform; (**b**) current waveform.

3. Experimental Result

3.1. Sterilization Effect by Pulsed Plasma to Microorganisms Living on the Food Surface

First, the sterilization effect to a food surface by pulsed plasma was investigated. Figure 5 shows the agar medium configuration and the position of *E. coli*. The agar medium of 15 mL was poured in the petri dish. The thickness of the agar medium was 2.36 mm. The diluted suspension of 100 μL, including *E. coli*, was applied on the agar medium. An applied condition of the plasma to the electrode is shown in Table 1. The discharge energies by the applied pulses calculated from waveforms, such as in Figure 4, are 32 mJ at 70 kV and 64 mJ at 85 kV.

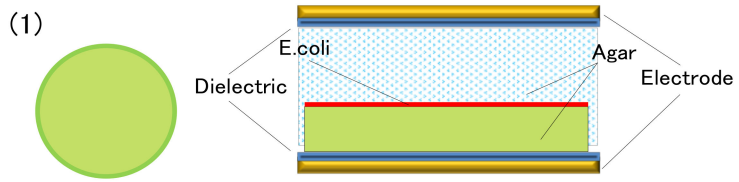


Figure 5. Agar medium configuration (1) and position of *E. coli*.

Table 1. Applied condition of pulsed voltage in plastic petri dish.

Parameter (Unit)	Value
Applied Voltage (kV)	70, 85
Repetition rate (pps)	10
Number of Applying pulses (shots)	5~100

Figure 6 shows the experimental result of the survival ratio on the surface of the agar medium in configuration (1) for the number of shots of applied pulses. The survival ratio decreased as the number of shots increased, and the high sterilization effect was obtained by higher applied voltage. In the applied voltage of 85 kV, the high sterilization effect was obtained by 75 shots. The microorganisms attached on the agar medium surface in the petri dish simulating the packaged food could be sterilized by the pulsed barrier discharge plasma. Figure 7 shows the survival ratio for the applied total discharge energy. It shows that the sterilization ratio on the surface of the agar medium depends on the applied total discharge energy. The discharge energy of about 4 J enables sterilizing all the *E. coli* on the surface.

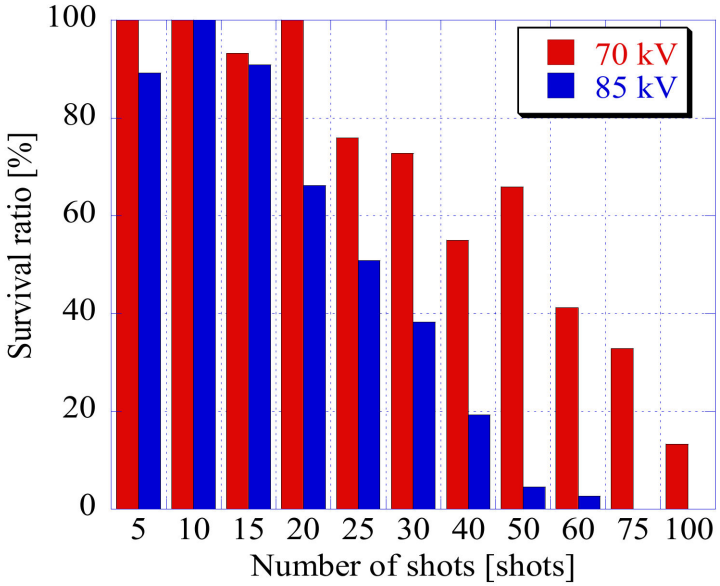


Figure 6. Survival ratios of *E. coli* on the surface of agar medium in configuration (1) by applying pulsed plasma.

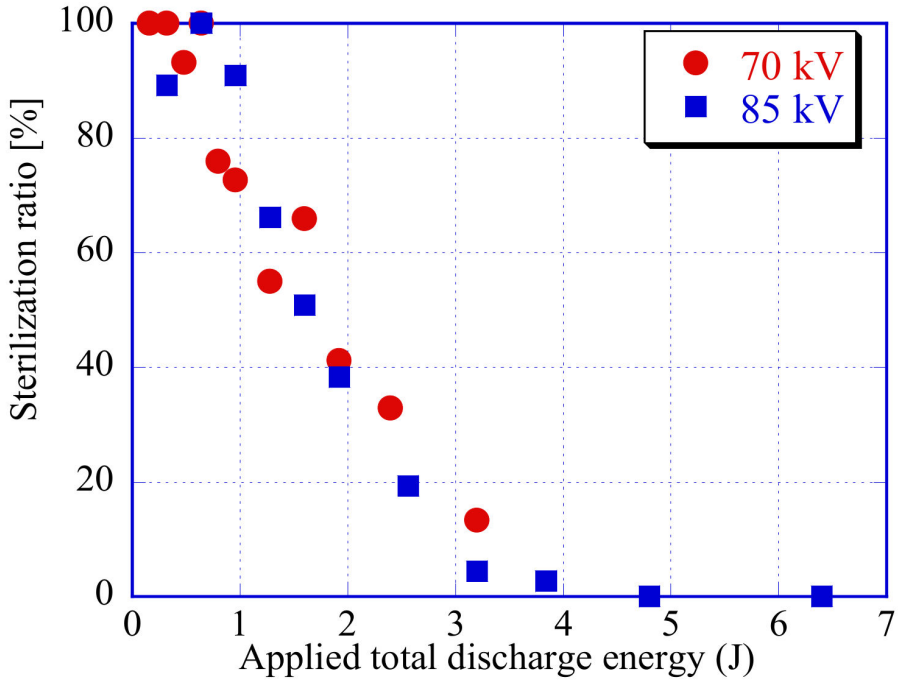


Figure 7. Survival ratios of *E. coli* on the surface of agar medium in configuration (1) for applied total discharge energy.

3.2. Investigation of Sterilization Effect by the Influx of the Active Species

In this section, the sterilization effect for between the overlapped pieces of the foods in the package has been investigated because it is a possibility that microorganisms attach on the pieces of the sliced foods. In this experiment, agar medium configurations of three types were investigated. Figure 8 shows those configurations. First (2) is the configuration that includes two pieces of agar medium stacked without a gap. This configuration simulates the overlapped food pieces without making the gap. The *E. coli* was applied to the agar medium in the same manner as in Figure 5. The upper agar medium is applied on the lower agar medium. The size of the upper agar medium is the diameter of φ 60 mm and the thickness of 2.36 mm. The different point of configuration (3) to configuration (2) is that the lower side of the upper agar medium has the hollow of the diameter of φ 32 mm and the height of 1.2 mm. This condition is simulated for the foods that include a hollow with air sealed by the overlapped pieces. The configuration of (4), (5) has the paths leading to the discharge area in the petri dish from the hollow. This condition simulated the foods including a hollow with air that is not sealed by the overlapped pieces. In the configuration of (4), (5), the lower agar medium is changed to the thickness of 6 mm, and the hollow of a diameter of φ 32 mm and a depth of 4.8 mm is made in it. On that, the two agar mediums that are the diameter of φ 60 mm and the thickness of 2.36 mm are stacked. However, the middle agar medium has a 5-mm- (4) or 15-mm-wide (5) slit at the center. This slit includes the paths leading to the discharge area in the petri dish from the hollow. The applied condition of the pulse voltage was the voltage of 85 kV, the repetition rate of 40 pps, and the number of applied pulses of 1000 and 5000 shots.

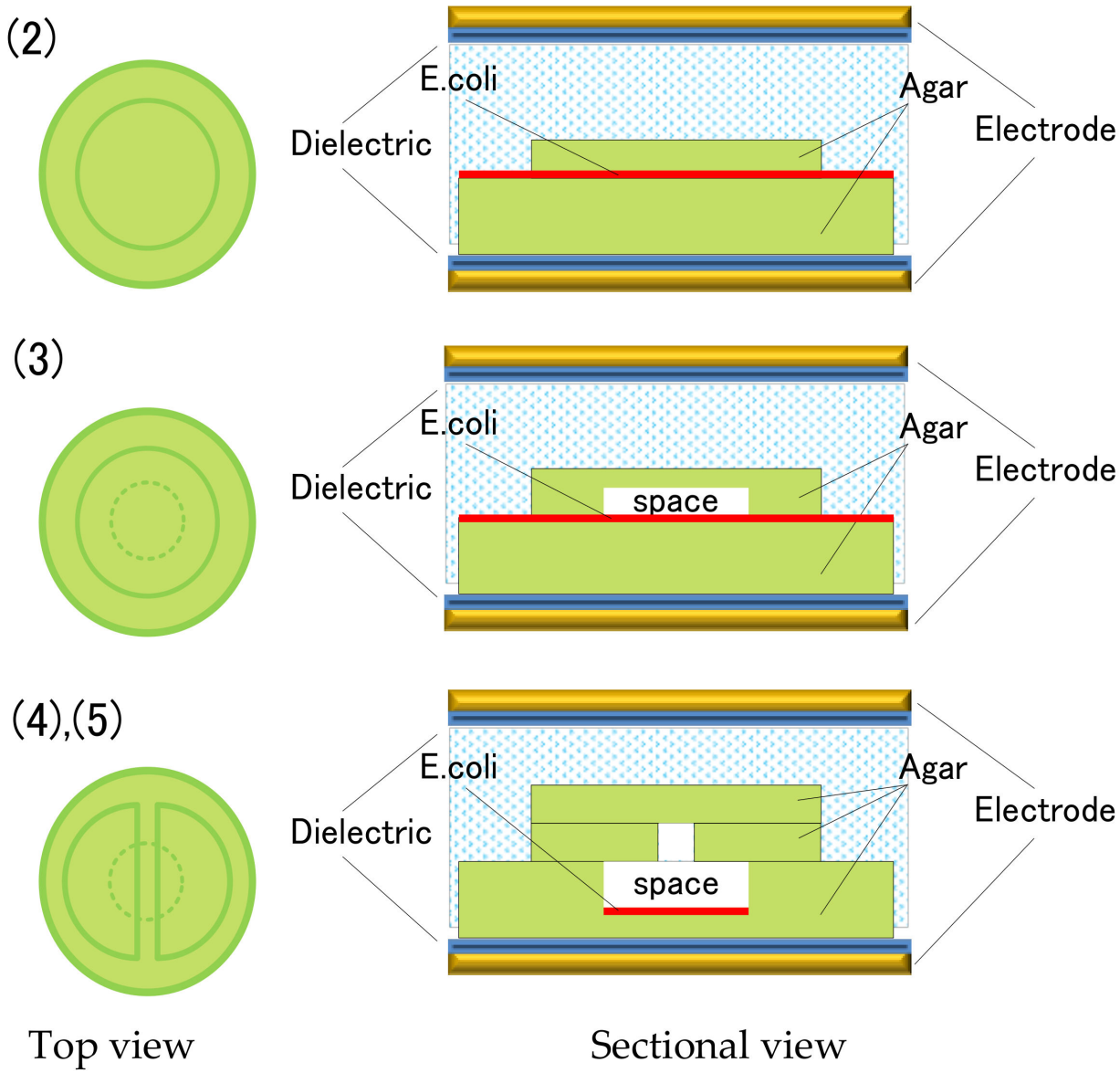


Figure 8. Agar medium configurations and positions of *E. coli*.

Figure 9 demonstrates the experimental results in configurations (2) to (5), and the sterilization results are summarized in Figure 10. In configuration (2), although the non-stacked area was sterilized, the stacked area was not sterilized. The reason is that the air for discharging does not exist in the stacked area. In configuration (3), which was made with the hollow, it goes without saying that the stacked area was not sterilized, although the non-stacked area was sterilized from the result of the configuration (2). However, even the hollow was not sterilized despite the existing air. The reason is thought to be that the agar mediums act as shields against applying the electric field to the hollow. Then, we simulated the distribution of the electric field in the hollow made by the agar mediums.

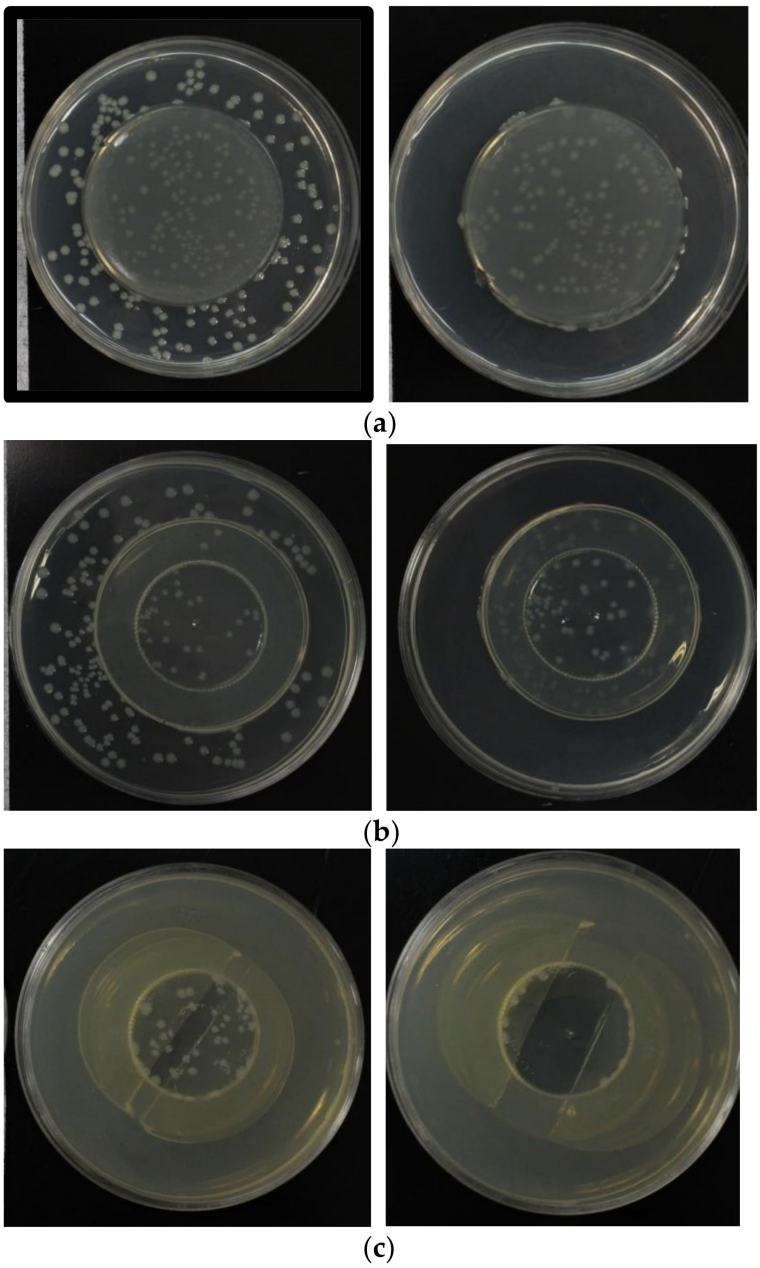


Figure 9. Appearances in each configuration. (**a**) Configuration (2); (**b**) configuration (3); (**c**) configuration (4), (5); The figures of left side are control. The figures of right side are after applying pulses.

The simulation software used was the Static-field Analysis Toolkit, (Field Precision LLC) [18]. This simulation software calculates using only either permittivity or conductivity. It cannot calculate using both permittivity and conductivity simultaneously. In the agar medium, conductance is more dominant than capacitance. On the other hand, in air and the material of the petri dish, capacitance is more dominant than conductance. Therefore, in this simulation, we used the magnitude of the complex relative permittivity of the agar medium, air, and petri dish for including both permittivity and conductivity for convenience. Table 2 shows the permittivity, conductivity, and magnitude of the complex relative permittivity for the materials used in the simulation. The complex relative permittivity, $\varepsilon_r{}^*$, is shown by:

$$\varepsilon_r{}^* = \varepsilon_r - j\frac{\sigma}{\omega\varepsilon_0} \quad (1)$$

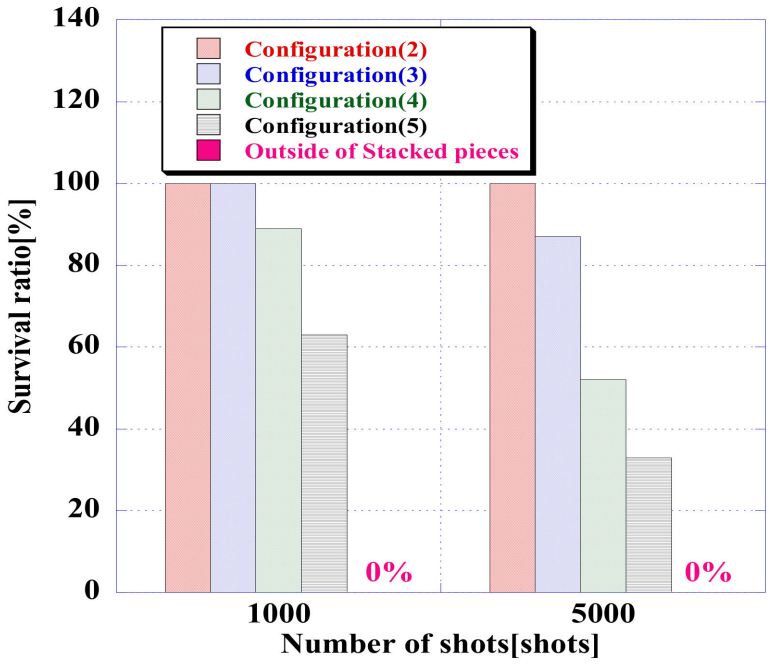


Figure 10. Survival ratios of *E. coli* in each configuration after applying each pulse number.

Here, ε_r is relative permittivity, σ is conductivity, ω is angular frequency, and ε_0 is permittivity of free space. In this simulation, the magnitude of complex relative permittivity was calculated from Equation (1), which, for convenience, is shown in the following:

$$\varepsilon_r{}^* = \sqrt{\varepsilon_r{}^2 + \left(\frac{\sigma}{\omega\varepsilon_0}\right)^2} \tag{2}$$

ω was calculated by using 5 MHz from the waveform in Figure 4.

Table 2. Magnitude of complex relative permittivity for simulation.

Material	Relative Permittivity	Conductivity [mS/cm]	Magnitude of Complex Relative Permittivity
Air	1	5×10^{-15}	1
Petri dish (polystyrene)	2.5	$<10^{-16}$	2.5
Agar medium	81	10	3596

Figure 11 shows the simulation result. The electric field strength in the hollow is almost the same as that of the agar medium. The estimated electric field strength of the hollow is about 3.1 kV/cm. This electric field strength cannot bring about the discharge in air. Therefore, configurations (4) and (5) are made to have the paths leading to the discharge area in the petri dish from the hollow. From Figure 10, it is found that configuration (5) with the slit of 15 mm can decrease the number of *E. coli* to two thirds at 1000 shots and one third at 5000 shots. Configuration (4) with the slit of 5 mm can decrease the number of *E. coli* to half at 5000 shots. The wider slit is easy to allow the ozone to pass to the hollow. It is thought that some ozone went to the hollow through the slit as the applied pulses increased.

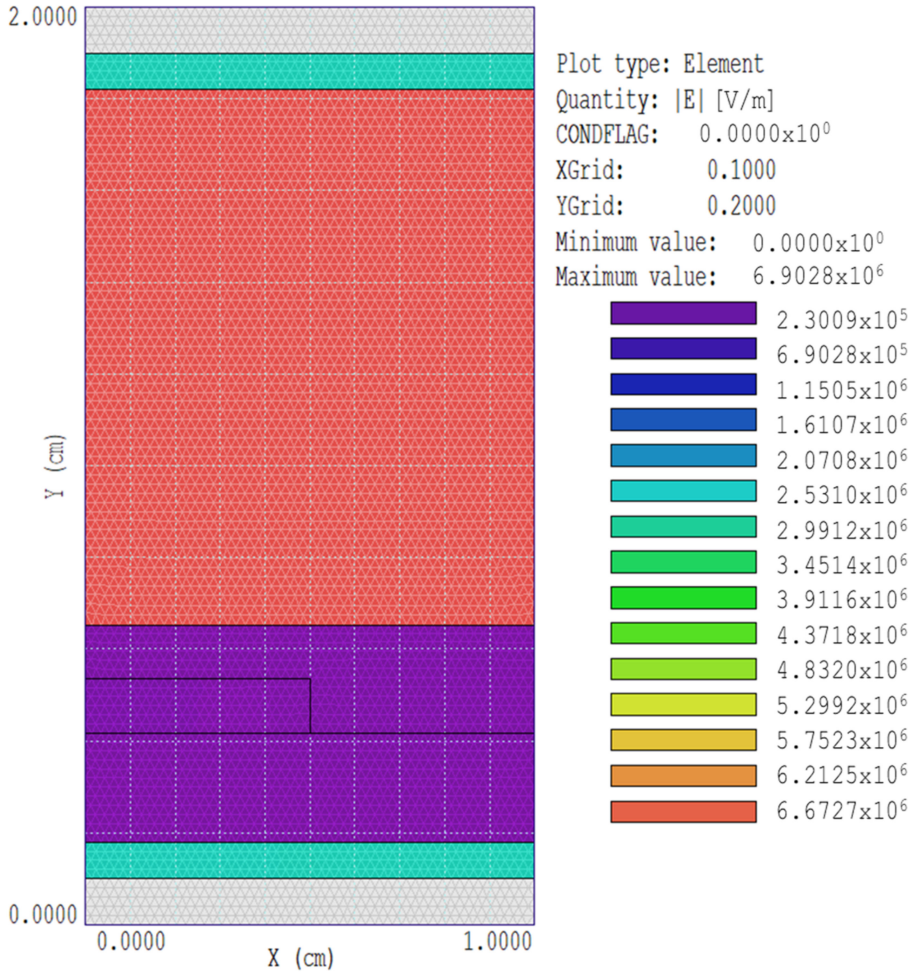


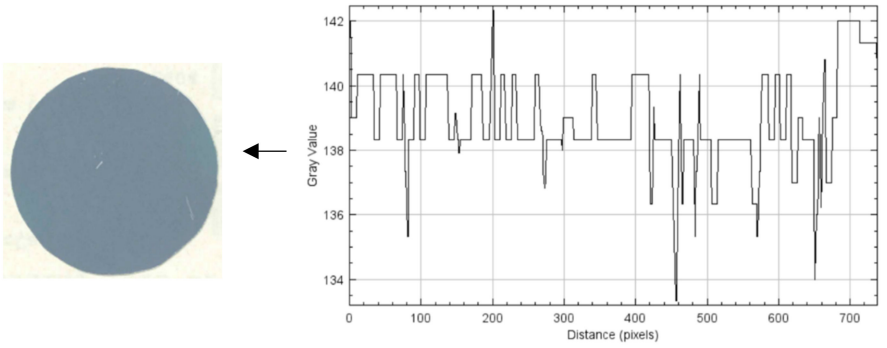
Figure 11. Simulation result of electric field distribution for configuration (3).

3.3. Investigation of Ozone Inflow to the Hollow through the Slit

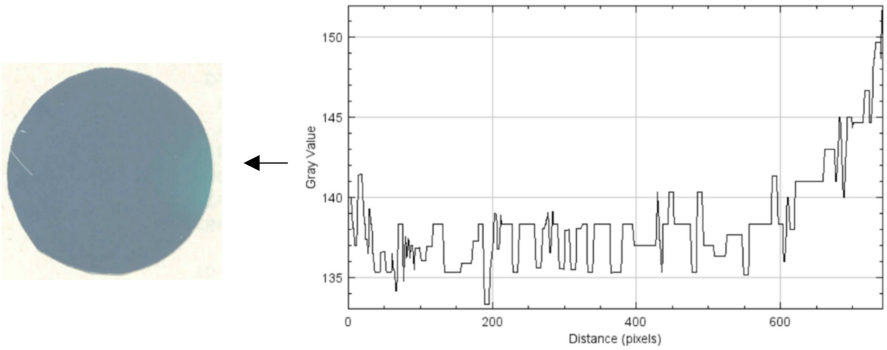
To confirm that some active species moved to the hollow through the slit, the Plasma Indicator™ (PLAZMARK™ for O_2 Cleaning: Sakura Color Products Co., Osaka, Japan) was used. The Plasma Indicator™ is a sheet that changes color from gray to light green by reacting to active species. The Plasma Indicator™ was put in the hollow of configuration (5) to detect the active species, as shown in Figure 12. In this experiment, the applied pulse voltage is 85 kV. Figure 13 shows the Plasma Indicator™ after applying the number of pulses of 500 shots, 1000 shots, 5000 shots, and 10,000 shots. Moreover, Figure 13 shows a gray scale value at the center of the slit in each sheet analyzed by ImageJ [19,20]. The color change of the Plasma Indicator™ at 500 shots was not detected. At 1000 shots, the right side of the Plasma Indicator™, which was located at the slit, slightly changed the color to light green. At 5000 and 10,000 shots, the right and left sides of the slit of the Plasma Indicator™ definitely changed the color to light green. Moreover, at 5000 and 10,000 shots, the gray scale values at the right and left sides are higher than those of the center, and the values become gradually smaller while moving closer to the center from both sides. The maximum gray scale value becomes high as the pulse number increases. These results demonstrate the active species, such as the ozone generated by the pulsed plasma enter through the paths leading from the discharge area to the hollow by diffusion. However, the sterilization effect at 5000 shots in the configuration (4) is roughly the same as that of 30 shots in Figure 6 for the surface sterilization by pulsed plasma. The sterilization effects for the foods with the hollow made by the overlapped pieces of the foods are not very high.

Figure 14 shows the ozone concentration in the petri dish for the elapsed time after the pulse application at agar medium configuration (1). The applied pulse voltage is 70 kV. The applied pulse number is 100 shots. The ozone concentration was measured by using the Kitagawa type gas sampler (KOMYO RIKAGAKU KOGYO K.K., AP-20). The ozone was generated 6.3 ppm immediately after the applied pulses. Then, the ozone concentration was decreased as the time elapsed and became zero at 60 s. The decrease in the ozone is fast in the presence of the agar medium. Therefore, if the ozone does not continue to be supplied in the slit by applying many pulses continuously, the ozone concentration does not increase and sterilization in the slit does not proceed.

Figure 12. Appearance of Plasma Indicator^{TM} put in configuration (5).



(**a**)



(**b**)

Figure 13. *Cont.*

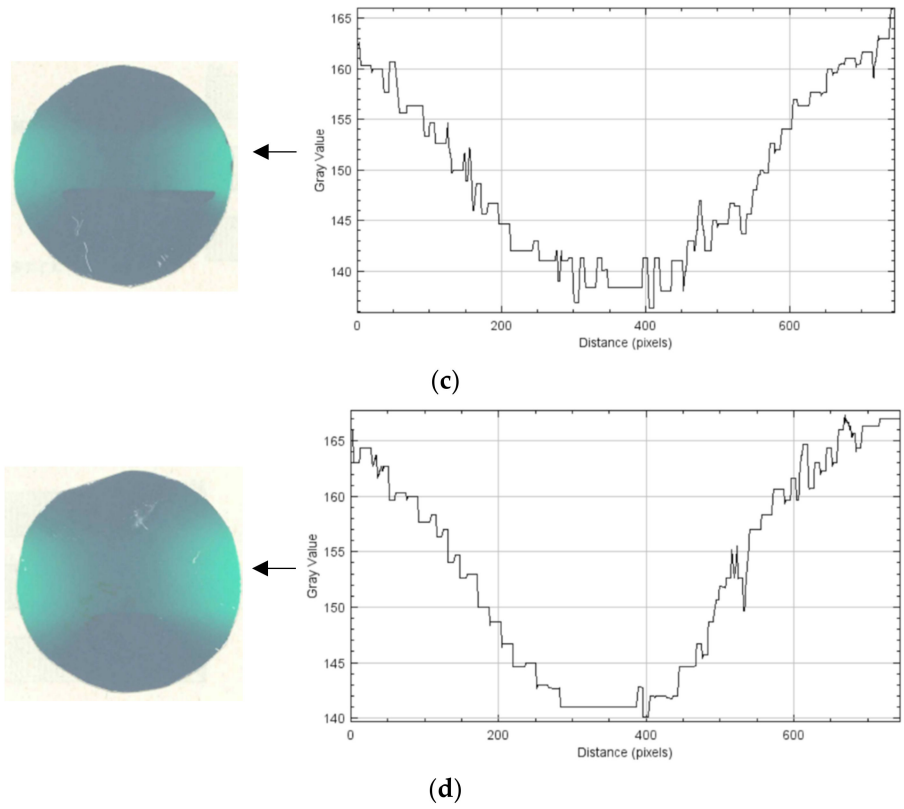


Figure 13. Color change of Plasma Indicator™ and gray scale value for each pulse number. (**a**) 500 shots; (**b**) 1000 shots; (**c**) 5000 shots; (**d**) 10,000 shots.

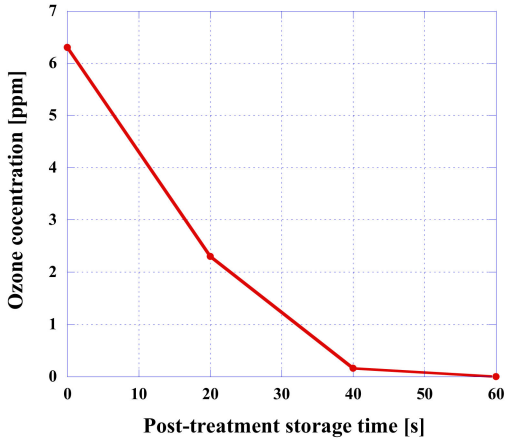


Figure 14. Ozone concentration in petri dish for elapsed time after pulse application. (Agar medium configuration (1); applied pulse voltage: 70 kV; applied pulse number: 100 shots).

3.4. Improvement of Sterilization Effect for the Hollow Made by Stacking the Pieces

To improve the sterilization effect for the hollow made by stacking the pieces, such as in configuration (3), the method for generating plasma in it was considered. The method is shown in configuration (6) of Figure 15. The improvement points in Figure 15 are below. Polyimide film (Kapton©: Toray DuPont, Tokyo, Japan), which has a lower dielectric constant and conductivity than agar because it causes electric field concentration inside the

hollow, is sandwiched between the upper and lower agar mediums covering the hollow by the upper agar medium, and the upper and lower agar mediums do not contact directly with each other. The thickness of the polyimide film is 126.7 μm. In addition, the height of the petri dish is changed to 8.6 mm so that the air space up to the lid of the petri dish from the top of the upper agar medium was eliminated. This change results in the increase in the applied electric field strength. The depth of the closed hollow is set from 0.5 mm to 4 mm in the experiment. In this experiment, the applied pulse voltage is 85 kV.

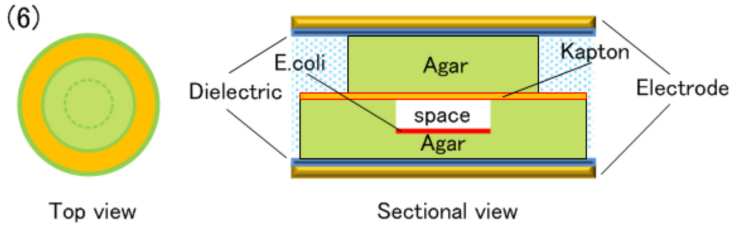
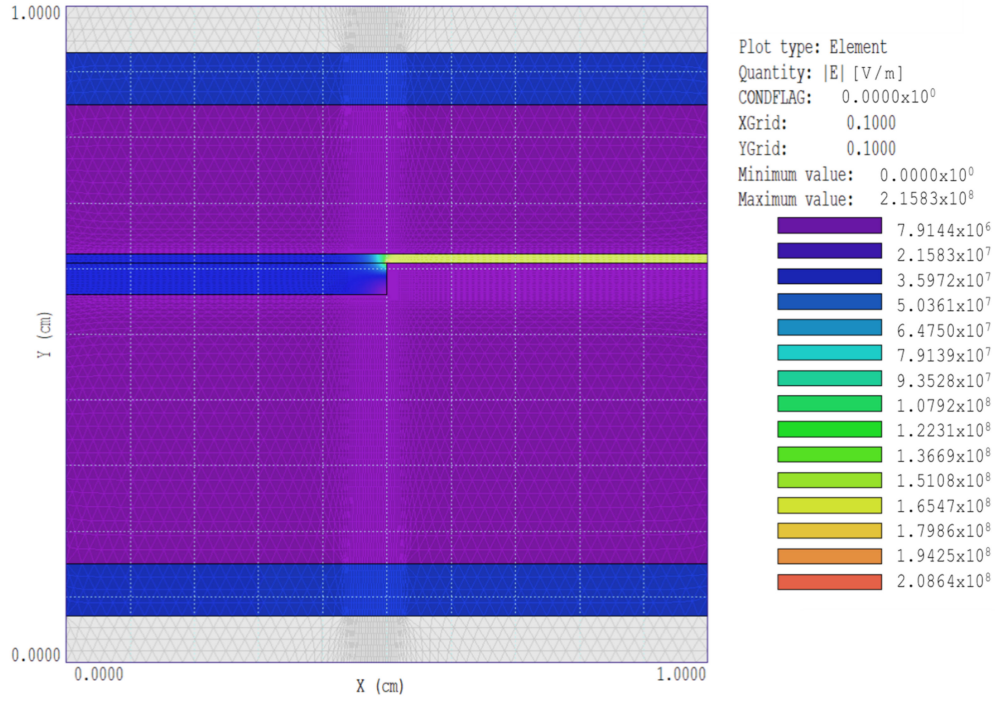


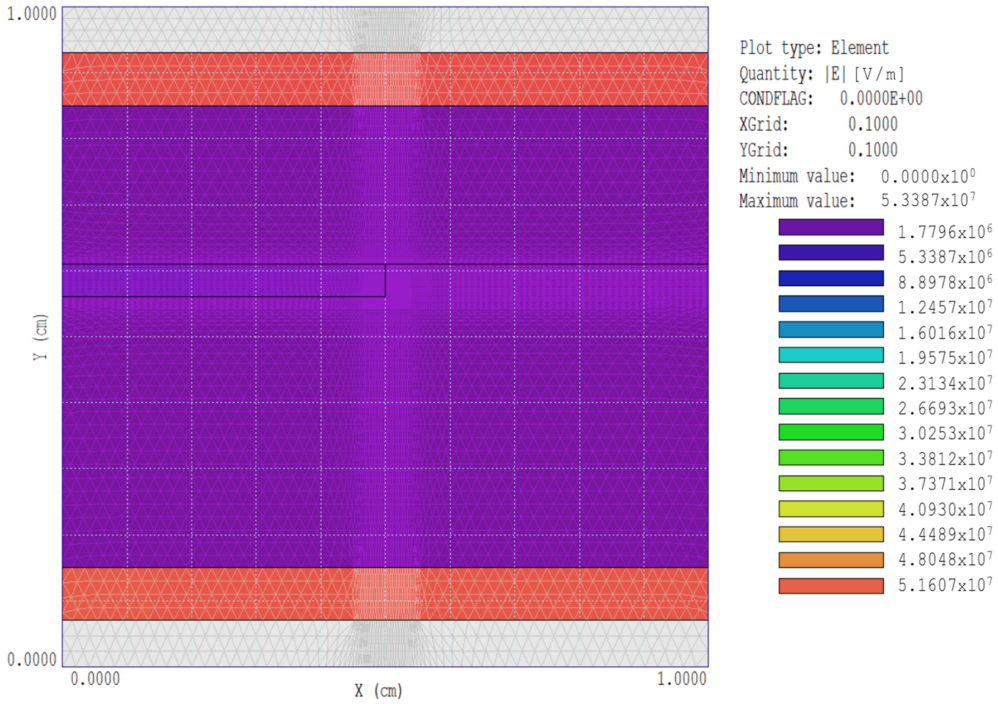
Figure 15. Agar medium configuration (6) with the polyimide film and positions of *E. coli*.

First, we simulated the distribution of the electric field in the hollow at the configuration (6). Figure 16 shows the simulation result at the depth of the hollow of 0.5 mm. Figure 16a shows the result with the polyimide film. Figure 16b shows the result without the polyimide film. The electric field strength in the hollow without the polyimide film is the same as that in the agar medium and is estimated to be about 10 kV/cm. In contrast, the electric field strength in the hollow with the polyimide film is much different from that in the agar medium and is estimated to be about 350 kV/cm. This electric field strength is able to cause the breakdown of air. Especially, the electric field strength is higher at the point of the triple junction of air, agar, and polyimide than elsewhere and is estimated to be about 800 kV/cm.



(**a**)

Figure 16. *Cont.*



(**b**)

Figure 16. Simulation result of electric field distribution for configuration (6). (**a**) With polyimide film; (**b**) without polyimide film.

Figure 17 shows the survival ratios of *E. coli* for the number of pulses at the depth of the hollow of 0.5 mm. It can be seen from the figure that the bactericidal effect is obtained at the configuration (6). It can also be seen that the number of *E. coli* decreases as the number of pulses increases. It is considered that *E. coli* was sterilized by the discharge occurring because the triple junction made by inserting the polyimide film enhanced the electric field strength.

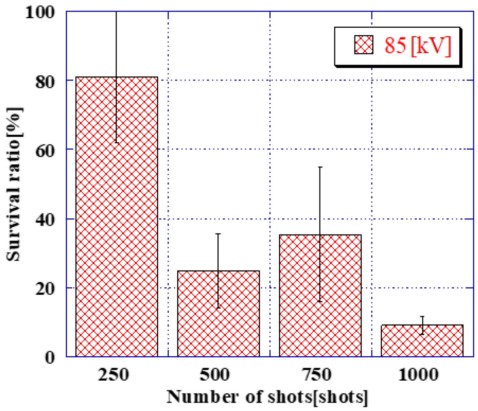


Figure 17. The survival ratios of *E. coli* after applying plasma of each pulse number in configuration (6) (depth of hollow: 0.5 mm).

Figure 18 shows the survival ratios when the depth of the hollow was changed. From the figure, it can be seen that the shallower depth of the hollow gives the higher bactericidal effect. However, when there is no hollow, the bactericidal effect is not obtained. From this result, it is considered that the hollow with air is indispensable for the generation of the plasma, but, if the hollow is too deep, the electric field does not concentrate in the

hollow and the bactericidal effect decreases. Figure 19 shows the electric field strength of the center of the bottom, where *E. coli* lives in the hollow, calculated by the simulation for each depth of hollow in configuration (6). As the depth of the hollow becomes deep, the electric field strength becomes low because the hollow area becomes wide, and the electric field does not concentrate in the hollow despite inserting the polyimide film. Figure 20 shows the relationship of the survival ratio and the electric field strength obtained from Figures 18 and 19. When the electric field strength exceeds 50 kV, pulse plasma is generated in the hollow, and it is thought that *E. coli* can be sterilized.

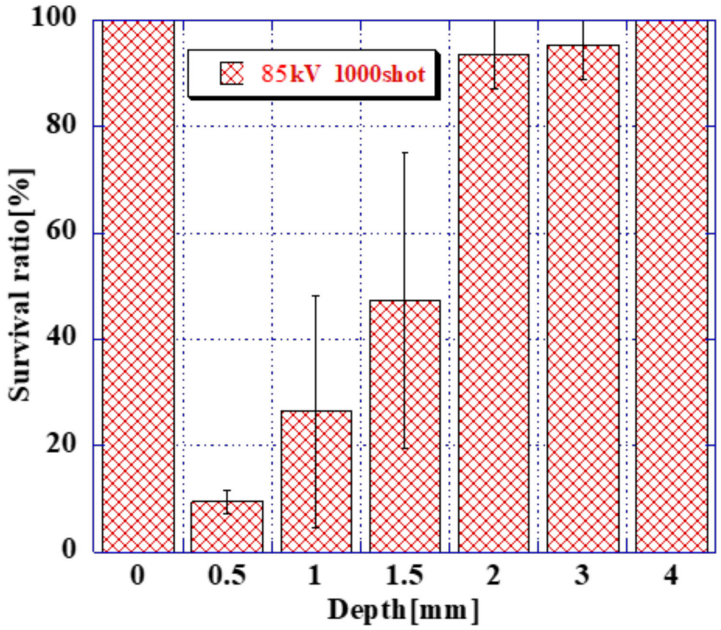


Figure 18. Survival ratios of *E. coli* for each depth of hollow in configuration (6).

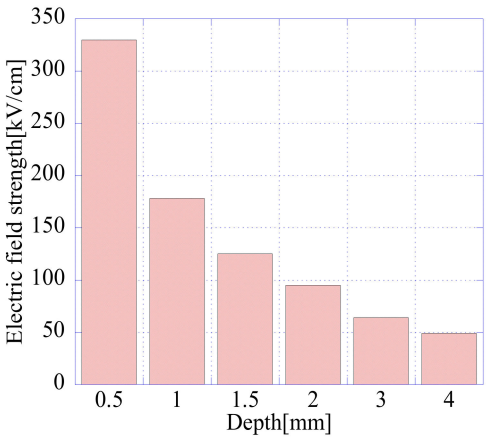


Figure 19. Electric field strength of the center of the bottom in the hollow calculated by the simulation for each depth of hollow in configuration (6).

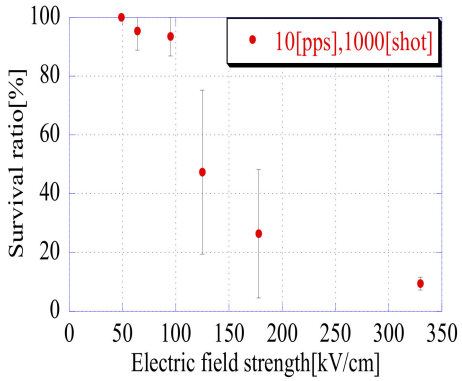


Figure 20. Survival ratio of *E. coli* for the electric field strength of the center of the bottom in the hollow in configuration (6).

4. Conclusions

In this paper, we focused on the sterilization of the overlapped pieces of food, such as salad and sashimi packaged in a container, which had not been investigated so far, and investigated the probability of sterilization by pulsed plasma. In the experiment, agar mediums were used for simulating the foods. The results are summarized below.

(1) The surface contacting with the plasma of the food packaged in the container can be easily sterilized.
(2) The sterilization ratio depends on the magnitude of the discharge energy of the plasma.
(3) When there is no air in the overlapped pieces, there is no bactericidal effect by the plasma.
(4) A hollow sealed completely by the pieces of food cannot be sterilized because it has a low electric field such that the plasma does not occur.
(5) Active species, such as ozone, generated by the plasma are diffusible, and, if a minute gap exists between foods, the active species can invade inside the gap and sterilize microorganisms. However, since those lifetimes are not so long, many pulses are necessary to generate a large number of the active species.
(6) The pulsed plasma can be generated in the hollow made by sandwiching the dielectric film between the pieces of food, and microorganisms can be sterilized.
(7) When the depth of the hollow made by sandwiching the dielectric film between the pieces of food becomes deep, the electric field in the hollow becomes low and the bactericidal effect becomes weak.

However, the bactericidal effect in the hollow and the slit is inferior to that on the surface, and the completely overlapped portion cannot be sterilized. In the future, based on these results, we aim to sterilize the parts where the fresh foods in the container completely overlap.

5. Patents

Japanese Patent No. 6751938 (P6751938); the title of the invention is: the sterilization method and the sterilization object using a pulsed plasma.

Author Contributions: Conceptualization, Y.M. and K.S.; methodology, Y.M. and K.S.; software, D.O.; validation, K.S., T.O., D.O. and Y.S.; formal analysis, Y.M.; investigation, K.S., T.O., D.O. and Y.S.; data curation, K.S.; writing—original draft preparation, K.S.; writing—review and editing, Y.M.; visualization, Y.M.; supervision, Y.M.; project administration, Y.M.; funding acquisition, Y.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by PROMOTING TECHNOLOGY TRANSFER AND INNOVATION, A-STEP (Adaptable and Seamless Technology Transfer Program through Target-driven R&D), grant number AS262Z02139K.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Pengcheng Cui for his help in performing this study.

Conflicts of Interest: The authors declare no conflict of interest.

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