

## Bacteria Sterilization by High Electric-Field Plasma without Discharges for Medical Applications

医療応用としての高電場プラズマによる殺菌

Fang-Yu Fan<sup>a,b,c</sup>, Chung-Ming Liu<sup>a,d,e</sup>, Chao-Chia Weng<sup>f</sup>, Yasushi Nishida<sup>a,g</sup>, and Keng-Liang Ou<sup>a,f,h</sup>

<sup>a</sup>Research Center for Biomedical Devices, Taipei Medical University, Taipei 110, Taiwan

<sup>b</sup>Graduated Institute of Dental Technology, Taipei Medical University, Taipei 110, Taiwan

<sup>c</sup>Department of Mechanical Engineering National Taiwan University

<sup>d</sup>Department of Chemical and Material Engineering, LungHwa University of Science and Technology, Taoyuan 306, Taiwan

<sup>e</sup>Graduate School of Engineering Technology, LungHwa University of Science and Technology, Taoyuan 306, Taiwan

<sup>f</sup>Graduated Institute of Biomedical Materials and Tissue Engineering, Taipei Medical University, Taipei 110, Taiwan

<sup>g</sup>Plasma and Space Science Center, National Cheng Kung University, Tainan 70101, Taiwan

<sup>h</sup>Research Center for Biomedical Implants and Microsurgery Devices, Taipei Medical University, Taipei 110, Taiwan

A comprehensive research for sterilizing bacteria by means of high electric-field plasma without apparent discharges between electrodes is carried out. The most common pathogens responsible for hospital-acquired infections are *Staphylococcus aureus* and *Escherichia coli*. The experimental results showed that these bacteria were sterilized up to 99 % *S. aureus* and 83 % *E. coli* within 60 second. The high electric-field plasma system can induce structural damage and ruptures on bacterial cells leading to the death of the bacteria.

### 1. Introduction

Using an effective sterilization method to control environment infections of human life has received much attention. Traditional sterilization technologies are the application of heat, chemical materials and/or irradiation to UV light or X-rays. Although these methods have been widely used in industries, healthcare and medical fields, but each method has its application limit. A high electric-field plasma (HEFP) technique in an atmospheric pressure has been developed to control and keep the environmental atmosphere in clean state, such as sterilization [1]. In this study, we will present the results of high electric-field plasma treatments of two types of bacteria. The sharp rise-time strong electric field formed in between the metal-plate electrodes shows no apparent discharges, but a few amount of electron currents are flowing. This system is free from the effects by ozone, NO<sub>x</sub>, OH\*(OH radical) or UV light emission. Those toxic materials do affect strongly to all of any materials or circumferences including human body.

### 2. Materials and Methods

In this study, the applied maximum voltage is 5.0 kV and the peak current is about 0.5-1.0 mA in the HEFP system. The antibacterial procedure was

according to JIS Z2801:2000 standard [2-3]. Two typical bacteria used as test strain for this experimental study are the Gram-positive *Staphylococcus aureus* (ATCC6538P: *S. aureus*) and Gram-negative *Escherichia coli* (BCRC11634: *E. coli*). These microbes are commonly used as research sites for environmental indicators.

A single colony was incubated for 18 h to 24 h at 37°C on nutrient agar plates (Difco, USA). The bacterial cells were standardized to concentration of  $2.5 \times 10^5 \sim 1.0 \times 10^6$  CFU/ml under sterile conditions by optical spectrophotometry (Thermo, USA). Portions of 10 µl of the standardized concentration suspensions were evenly spread on the dielectric glass substrates. The each sample are placing/inserting at the center of the metal-plate electrodes with 1.2 mm gap spaces. The samples were treated by HEFP with four different treatment time of 0, 15, 30, 45 and 60 second, in which each condition had replication for three times. The number of colonies was calculated to the antibacterial rate (AR). To investigate the effects of damages caused by HEFP treatment on bacteria cells, the Scanning electron micrographs (SEM) was used to inspect morphologies of *S. aureus* and *E. coli* cells.

### 3. Results and discussion

The SEM images of *S. aureus* is shown in Fig. 1 at each time duration after HEFP treatment. These images indicate that the outer membrane ruptures and splits matrix and distortion of the dead cells after plasma treatment. The SEM observation on *E. coli* in Fig. 2 also shows similar results. The HEFP treatment is verified, showing very effective for bacteria sterilization.

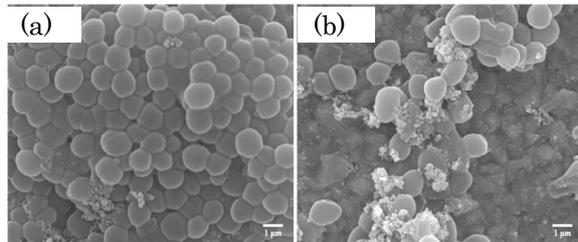


Fig.1. The results of Scanning electron micrographs of *S. aureus* at different time duration in HEFP treatment. (a) 0 s (b) 60 s.

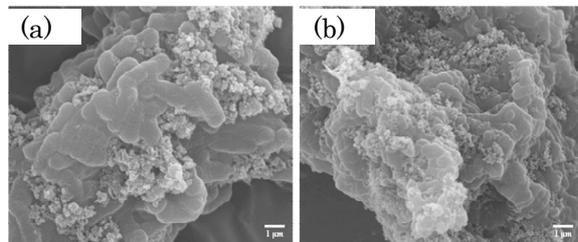


Fig.2. The results of Scanning electron micrographs of *E. coli* at different time duration in HEFP treatment. (a) 0 s (b) 60 s.

The bacterial of *S. aureus* (gram positive) has 3 layers as cell membrane, including plasma membrane, periplasmic space and peptidoglycan from inside of the cell. In the case of *E. coli*, however, it has an outer membrane which acts as a protective layer for the Gram-negative microbes [4-5]. Therefore, it may be rather easier to destroy the cell membrane in case of *S. aureus*. However, it is of great importance for practical systems for the analysis that the antibacterial rate (AR) could be determined by Eq. (1) as recognized for antibacterial effect.

$$AR(\%) = 100 \times \frac{N_{control} - N_{treated}}{N_{control}} \quad (1)$$

Here,  $N_{control}$  is number of bacteria adhered on untreated samples after 24 h incubation, and  $N_{treated}$  is number of bacteria adhered on treated samples after 24 h incubation.

The survival rates of these bacteria after undergoing different treatment times are shown in Fig.3. Results clearly show that continuous increase of exposure time to HEFP perfectly led to the decrease of survival rate of these microbes. The antibacterial rate (AR) could be simplified for

different timing on HEFP sterilization. Therefore, the percentage for an effective sterilization could be achieved at 30 second of treatment. However, *S. aureus* shows better sterilization than *E. coli*, which could be seen clearly after 60-second treatment, i.e. 99 % of initial *S. aureus* is effected by HEFP treatment. It was noted that the response of *S. aureus* was significant than *E. coli*.

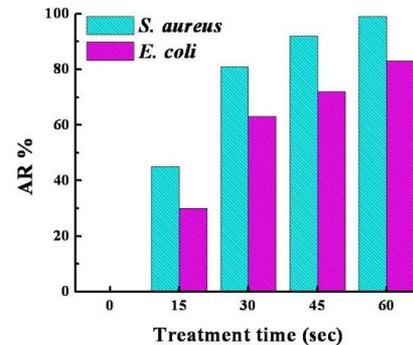


Fig.3. Antibacterial rate of *S. aureus* and *E. coli* after different treatment time.

#### 4. Conclusions

This experimental analysis has been carried out as part of a larger effort aimed for determining and understanding the fundamental processes involved in new technique of HEFP sterilization. From our results on the experiments, effective measures on the sterilization technique were clarified for both Gram-positive and Gram-negative bacteria.

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