OPTIMIZATION OF ELECTRIC FIELD FREQUENCY ON DIELECTROPHORETIC IMPEDANCE MEASUREMENT METHOD FOR ORAL BACTERIA DETECTION

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Abstract: A simple and rapid bacteria detection device for on-site evaluation of oral hygiene in hospitals and clinics was demonstrated. The developed device utilizes dielectrophoretic impedance measurement (DEPIM) method. We integrated a micro electrode chip on which bacteria were captured by dielectrophoresis (DEP), an AC voltage source to induce DEP force, and an impedance measurement circuit to a portable instrument that enables rapid and automated oral bacterial inspection in hospitals and clinics. Special considerations have been made on effects of high electrical conductivity of oral samples on DEP force and DEPIM results. It was shown experimentally and theoretically that using a higher electric field frequency for the DEP bacteria trap and the impedance measurement could realize DEPIM application to bacteria inspection from oral samples with higher conductivity. Based on these investigations, we optimized the frequency condition of the DEPIM suitable for inspecting an oral sample along with the design and development of a portable DEPIM apparatus for on-site inspection of oral bacteria.

1 INTRODUCTION

Microbiological infectious disease of the oral cavity is one of the matters for greatest concern since the relationship between influenza, pneumonia and oral bacteria, so that accurate evaluation of the amount of oral bacteria as a level of oral hygiene is required in order to prevent influenza (Abe *et al*., 2006a) and aspiration pneumonia (Abe *et al*., 2006b). In this study, a simple and rapid bacteria detection device for on-site evaluation of oral hygiene in hospitals and clinics was demonstrated. The developed device utilizes dielectrophoretic impedance measurement (DEPIM) method (Suehiro *et al*., 1999). Bacteria suspended in a solution is trapped at the gap of interdigitated microelectrode by positive dielectrophoresis (DEP), simultaneously, temporal change of capacitance of the electrode is measured. Bacteria concentration is calculated based on a tangent slope of capacitance change. Effect of high conductivity of oral samples on DEP force and DEPIM results was experimentally and theoretically validated.

2 MATERIAL AND METHODS

2.1 Electrodes

Two different electrode configurations were used. A smooth interdigitated electrode system was employed in all the DEPIM experiments because this type of electrode configuration is suitable for accurate impedance measurement (Suehiro *et al*., 1999). The smooth interdigitated electrode arrays of gold were patterned on a polycarbonate substrate by

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a laser ablation technique. Each microelectrode strip had a 5 μm gap in which cells were trapped and formed into pearl-chains by positive DEP. On the other hand, a castellated electrode configuration (Wang et al., 1993) was employed for the visual observation of the cell collection process using positive DEP. The castellated electrode arrays of chrome were patterned on a glass substrate by photolithography technique, and the microelectrode was surrounded by a silicon rubber spacer to form a chamber in which 22 μl of bacterial suspension liquid was stored.

2.2 DEP Observation Equipment

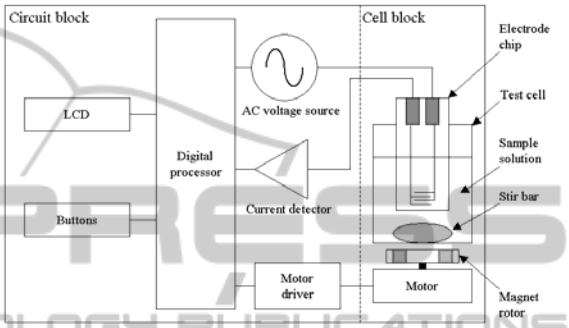
The cell suspension liquid was stored in a reservoir tank and circularly fed to the test chamber using a peristaltic pump (Suehiro et al., 1999). Sinusoidal AC voltage was generated by a function generator (WF 1945, NF Corporation, Japan) and applied to the electrode system. Visual observation of DEP was conducted using an inverted microscope (BX-51, OLYMPUS, Japan) and a CCD digital camera (C-5060Z, OLYMPUS, Japan). The flow rate of the cell suspension liquid fed by the peristaltic pump was 2.1 ml/min, and the amplitude of the applied voltage was 10.0 V peak–peak respectively, which were found to be appropriate conditions for the observation of positive DEP in the preliminary tests.

2.3 DEPIM Equipment

Fig. 1 shows a block diagram and a photographs of the newly designed and developed DEPIM apparatus and electrode chip. To enable rapid and automated bacterial inspection in hospitals and clinics, the apparatus was designed as a portable instrument to enable stand-alone measurement without any other instruments or cables.

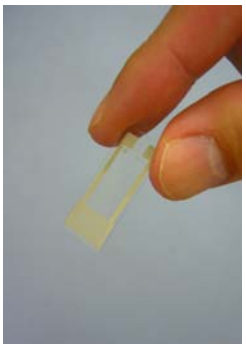
The AC voltage source generates AC voltage, which energizes the interdigitated electrode to generate positive DEP force. Amplitude of the applied voltage was 5.0 V peak–peak. AC current flowing through the electrode is measured by the current detector. The processor calculates the electrode capacitance from the amplitudes of the applied AC voltage and detected current, and the phase difference between the two components. The sequential measurement is carried out for 20 s, and temporal variation of the electrode capacitance is stored, then a tangent slope of capacitance change is calculated in order to estimate bacteria concentration, which has a linear relationship with the slope.

In the test cell, 5 ml of bacterial suspension is stored, in which the smooth interdigitated electrode is immersed. The electrode chip is connected to the AC voltage source and current detector. A magnetic stirrer continuously generates a circular flow in the test cell to enhance the DEP trapping of bacteria. Impedance values measured by the DEPIM apparatus were calibrated using a dummy load (a parallel connection of resistance and capacitance with known values), as well as a buffer with known conductivity.



(a)

(b)



(c)

Figure 1: The block diagram (a) and photographs of a newly designed portable DEPIM apparatus (b) and an electrode chip (c).

2.4 Bacteria Samples

For observation of the DEP trapping process and optimization of DEPIM conditions, Escherichia coli (E. coli) strain K-12 (NBRC3301), which have a high growth rate and have been successfully employed in previous works (Suehiro et al., 1999), were employed as a dummy of oral bacteria in order to improve efficiency of experiments. E. coli were incubated on agar plates for 24 hours. Before each measurement, cells were harvested from the agar and suspended in a 0.1 M mannitol solution. After several washings by centrifugation, they were finally resuspended in a 0.1 M mannitol solution (1 μS/cm)

at various diluted concentrations as determined by a colony counting method.

Conductivity of the mannitor solution was adjusted range up to 50 μS/cm by dissolving sodium chloride to simulate mixing of human saliva. This value corresponds to be roughly 150 times diluted human saliva by deionized water (Neyraud et al., 2009), and bacteria concentration of human saliva (Abe et al., 2008) at the dilution strength will be detected by DEPIM method (Suehiro et al., 1999).

3 RESULTS

3.1 Observation of DEP Trapping Process of Bacteria

Photographs of the DEP collection of *E. coli* are shown in Fig. 2. The DEP collection observations were made at two different electric field frequencies of 100 kHz (Fig. 2a) and 800 kHz (Fig. 2b), and conductivity of the suspending medium of 50 μS/cm. Bacteria were not trapped at 100 kHz, while some bacteria were captured at 800 kHz. These observation results suggest that positive DEP force exerted on the bacteria becomes weak with increased conductivity at the 100 kHz frequency.

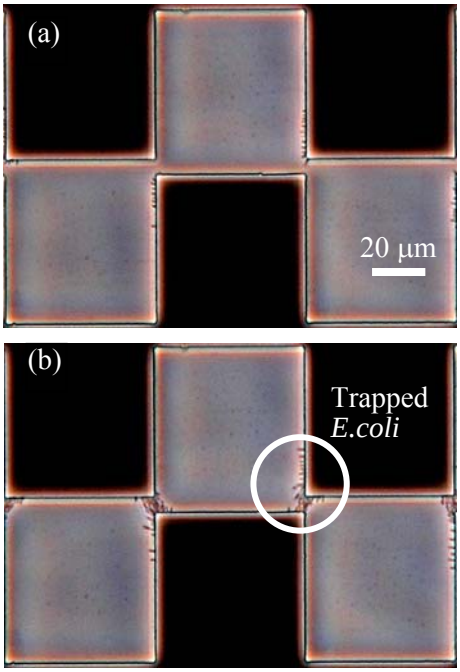


Figure 2: DEP collection process of *E. coli* at medium conductivity of 50 μS/cm and at frequency of 100 kHz (a) and 800 kHz (b).

3.2 DEPIM Measurement using E. coli Samples

DEPIM experiments were conducted in this range of conditions for 1 μS/cm and 50 μS/cm. Fig. 3 shows temporal variation of the electrode capacitance increment measured with *E. coli* at $5x10^6$ CFU/ml (at 100 kHz) and $2x10^7$ CFU/ml (at 800 kHz). Capacitance increase is due to the presence of bacteria that are trapped and enriched in the electrode gap. At a frequency of 100 kHz, the capacitance increase rate in the case of 25 μS/cm was obviously lowered in comparison with 1 μS/cm (Fig. 3a). However, at a higher frequency of 800 kHz, the temporal change of capacitance was almost the same for both the conductivities of 1 μS/cm and 50 μS/cm (Fig. 3b).

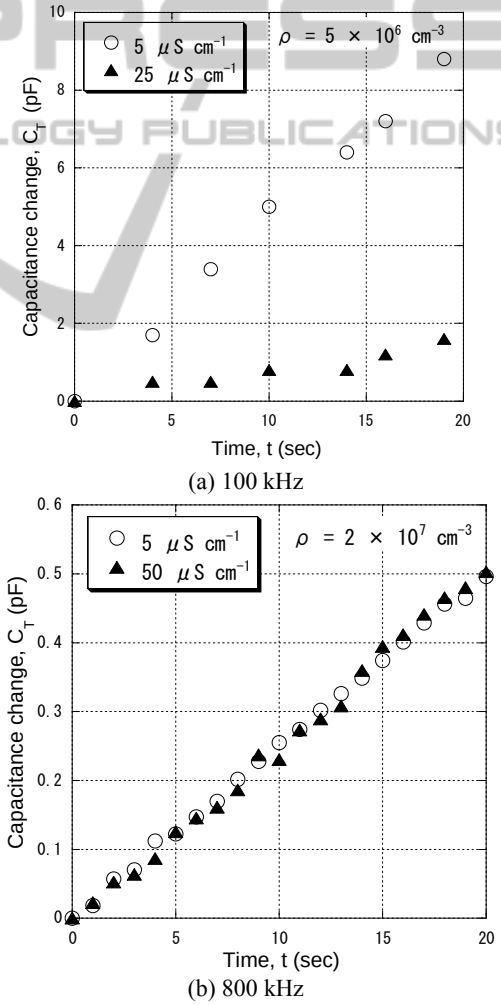


Figure 3: DEPIM results measured with *E. coli*. at frequency of 100 kHz (a), and 800 kHz (b).

4 DISCUSSION

The DEP force acting on a spherical particle of radius r suspended in a medium of permittivity □s is given by (Jones, 1995)

$$F_{DEP} = 2\pi r^3 \varepsilon_s \, \mathrm{Re}[K(\omega)] \nabla E^2 \quad (1)$$

where E is the magnitude (RMS) of the applied field and Re[K(ω)] is the real component of the Clausius–Mossotti factor given by

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_s^*}{\varepsilon_p^* + 2\varepsilon_s^*} \quad (2)$$

where ε_p^* and ε_s^* are the complex permittivity of the particle and surrounding medium, respectively. For a real dielectric, the complex permittivity is defined as

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \quad (3)$$

where ε is the permittivity and s is the conductivity of the dielectric and ω is the angular frequency of the applied field.

An example of a theoretical prediction of the suspension medium conductivity dependency of parameter Re[K(ω)] is shown in Fig. 4. One E. coli cell is modeled as a dielectric sphere covered by shells. The shells represent the cytoplasmic membrane and the sphere covered by the shells represents the cytoplasm (Huang et al., 1992). Parameter values of E. coli are determined referring to the referenced literature (Llamas et al., 1998). Fig. 4 indicates that Re[K(ω)] or the DEP force decreases with increases in the medium conductivity σs at a lower field frequency. When the medium conductivity increases from the initial value of 1 to 50 μS/cm, DEP changes from positive-DEP to negative-DEP at the field frequency of 100 kHz. This suggests that *E.coli* cells are not captured at the electrode gap by DEP under the condition of 50 μS/cm. On the other hand, the DEP force is hardly dependent on σ_s at 800 kHz. The theoretical calculations agree well with the experimental results shown in Fig. 2 where DEP collection of *E.coli* is observed only for low medium conductivity (1 μS/cm) at 100 kHz but no clear differences are observed with a rise in medium conductivity until 50 μS/cm at 800 kHz.

These results indicate that frequency of 800 kHz is more appropriate than 100 kHz for DEPIM measurement of sample with high medium electrical conductivity, σ_s.

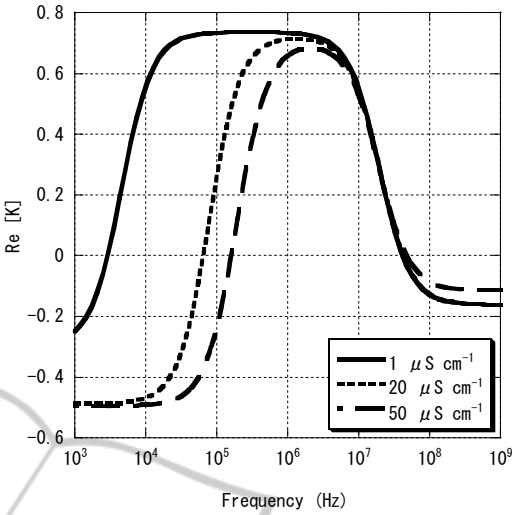


Figure 4: Theoretical prediction of the external medium conductivity σ_s dependency of Re[K(ω)] spectra.

5 CONCLUSIONS

In this study, we have described the optimization of AC electric field frequency in the DEPIM method to enhance the measurable range of conductivity of the sample solution to adapt the DEPIM method for the inspection of bacteria obtained from the human oral cavity. Observation and theoretical calculation of DEP, and DEPIM measurement was carried out. From these results, it was shown that higher field frequency is more suitable condition for bacterial sample that has higher electrical conductivity of solution. Consequently, it was demonstrated that the developed portable DEPIM apparatus is useful in the on-site evaluation of the bacterial contamination of clinical samples from the oral cavity for quantitative evaluation of oral hygiene to prevent influenza and aspiration pneumonia. In addition, the developed apparatus will be applied to other fields in which the investigation of the sample including ionic substances is necessary, for example, any clinical samples besides those taken from the oral cavity, as well aw fields relating to the environment and the food industry.

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