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Demonstration of New Microelectrode Design to Enhance Sensitivity of Dielectrophoretic Impedance Measurement

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Abstract—Dielectrophoretic impedance measurement (DEPIM) is a method using a microelectrode for measuring a target suspended in a solution by combining dielectrophoretic (DEP) trapping of the target and simultaneous measurement of impedance varied with the trapping. DEPIM can be utilized to detect bacteria and viruses without any chemical additive. Since DEPIM efficiency depends on the DEP trapping dominated by the microelectrode shape. This study aimed to demonstrate a new microelectrode design to increase bacteria detection efficiency instead of the conventional interdigitated microelectrode. The microelectrode having a serpentine gap as a sinusoidal waveform profile was proposed. The new microelectrode was compared with the interdigitated one in numerical simulation and experiments. The numerical simulation demonstrates that the serpentine microelectrode traps all the targets in a laminar flow in a microchannel. In contrast, the interdigitated one placed parallel to the microchannel doesn't because the target flowing away from the edges of the microelectrode cannot be trapped. The experiments using three kinds of bacteria were carried out. It was demonstrated that the serpentine microelectrode represented a sensitivity 10^4 times higher than the interdigitated one when a small amount of the sample solution was tested for a short time (1 min).

Index Terms—Bacteria detection, Dielectrophoresis, Impedance measurement, Serpentine microelectrode

I. INTRODUCTION

Dielectrophoresis (DEP) is a kind of electrokinetic motion of a dielectric particle under a non-uniform electric field [1]. DEP depends on the polarizability of the dielectric and the gradients of the electric field ($\nabla|E|^2$, where E is the electric field). If the dielectric has higher polarizability than the surrounding medium, it moves to the high electric field region, calling positive DEP. Otherwise, the less polarizability dielectric moves to a weak electric field region, calling negative DEP. The polarization depends on the complex permittivity of the dielectric, meaning DEP depends on not only the nature of the target but also the conductivity and permittivity of the surrounding medium and the applied frequency. DEP has been utilized to manipulate small objects such as bacteria, viruses, DNA, and micro and nano-sized particles.

We have developed rapid and simple bacteria and virus detection method based on DEP trapping of the target, called dielectrophoretic impedance measurement (DEPIM) [2], [3]. In DEPIM, the target bacteria are trapped to a microelectrode by positive DEP, and simultaneously, the change of the microelectrode impedance caused by the trapping is monitored to quantify the trapped bacteria. Recently, DEPIM has been utilized to detect DNA amplified by polymerase chain reaction (PCR) by attaching it to dielectric microbeads [4].

Although DEPIM is very simple and effective in detecting bacteria, several arrangements of DEPIM have been demonstrated to enhance the sensitivity of bacteria detection. For example, placing an additional microelectrode on a top of a microchannel upstream from the main microelectrode placed on a bottom of a microchannel [5]. A voltage with a lower frequency was applied to the additional microelectrode to generate negative DEP to push the target bacteria from the top to the bottom of the microchannel, where the positive DEP occurs on the targets effectively. It increased the sensitivity due to the increase of the trapping. As the other approach, we applied the relatively high voltage (20 V_{PP}) of a rectangular waveform to the trapped bacteria to disrupt their membrane [6]. After disrupting the membrane, cytoplasm components, such as ions released. They represent an increase in the conductivity of the microelectrode. We call it electropermeabilization-assisted DEPIM (EPA-DEPIM). EPA-DEPIM makes a signal from trapped bacteria whose amount is not enough to represent measurable impedance change.

In this study, another approach to enhance the sensitivity of DEPIM was considered. We focused on the shape of the microelectrode. An interdigitated microelectrode consisting of several straight lines placed and connected in parallel has been used for DEPIM. In our standard setup, the interdigitated microelectrode consisting of four fingers with three gaps is employed. Here, we proposed a microelectrode having a serpentine gap shaping a sinusoidal waveform. The serpentine microelectrode was designed to capture the bacteria in laminar flow generated in a microchannel. The

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effectiveness of the microelectrode was demonstrated by numerical simulation and experiments to detect three kinds of bacteria.

II. ELECTRODE DESIGN AND NUMERICAL SIMULATION

Fig. 1 shows images of the conventional interdigitated and new serpentine microelectrodes. The new microelectrode was designed especially for DEPIM with a microchannel. The flow in the microchannel forms the laminar flow due to its small dimension. Suppose the interdigitated microelectrode is placed parallel to the microchannel. In that case, it is difficult to trap the targets flowing far from the microelectrode edges where the electric field is the strongest, such as above the middle of the metal regions, because the positive DEP causing on the targets dramatically decrease with the distance from the edges in principle. If the interdigitated microelectrode is placed perpendicular to the microchannel, the capacity of the gap to trap the target becomes small.

The serpentine electrode having a sinusoidal waveform gap was designed to cover the whole width of the microchannel. In this design, a single gap was utilized. To cover the entire width by the interdigitated microelectrode, we need to increase the number of gaps and decrease the width of the metal region. The former and the latter will increase the total capacitance and inductance, respectively. Generally, the capacitance and inductance of the thin metal fabricated on an insulator increase with increasing the gap length and decreasing the metal width, respectively. The capacitance increase causes it to be difficult measuring the impedance change during the DEP trapping. The inductance increase causes the voltage drop in the metal electrodes.

Numerical simulation was carried out using COMSOL ver. 6.0. Trajectories of particles, 10 μm in diameter, having positive DEP property, flowing in a microchannel, 200 μm in width, were calculated. The interdigitated microelectrode was placed parallel to the microchannel and had four gaps of 5 μm. The serpentine microelectrode had a sinusoidal waveform of 5 μm gap, and its wavelength was set to 0.5 mm. The particles were placed 50 μm above the bottom of the microchannel where the microelectrodes were placed. The normal flow velocity and the applied voltage were set to 5 mm/sec and 20 V, respectively. Here, the applied voltage in the simulation is higher than that applied to the experiments to distinguish

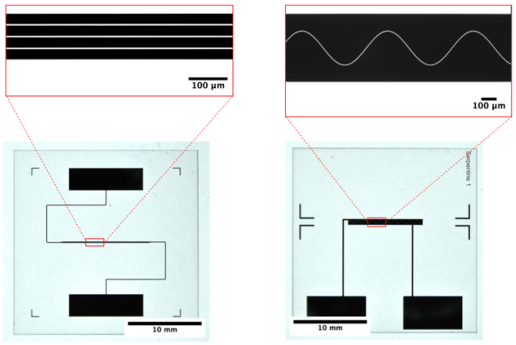

Fig. 1. Images of the interdigitated (a) and serpentine (b) microelectrodes. The microelectrodes were made of chromium and were fabricated on glass substrates.

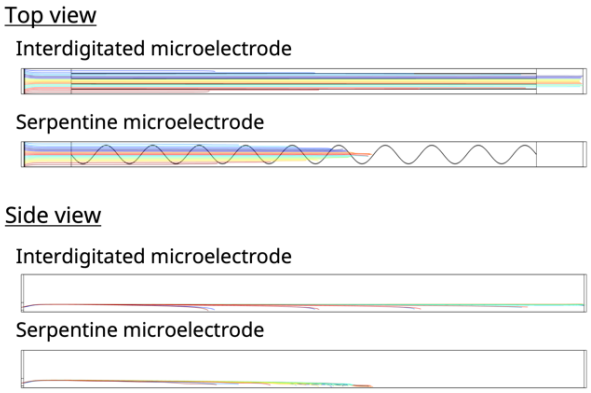

Fig. 2. Simulated particle trajectories in the case of the interdigitated and the serpentine microelectrodes for trapping the targets in the laminar flow in the microchannel. Each colored line indicates the trajectory of each particle. The total particle number was 20.

the effect of the serpentine microelectrode from that of the interdigitated one.

Fig. 2 shows the calculation results. In the case of the interdigitated microelectrode (Fig. 2 (a)), only the particles flowing around the edges of the microelectrode were trapped, while the others were not. As mentioned above, this is because the flow in the microchannel becomes the laminar flow, and the DEP force depends on the distance from the edges. On the other hand, when the serpentine microelectrode was used, all particles were trapped. The particles experienced positive DEP force, which pushed them to the bottom when they flowed over the gap. After across the gap at appropriate times, the particles were trapped to the gap because the particles were introduced into the regions with enough positive DEP occurring. Because the sinusoidal waveform covers the entire microchannel width, the particles flowing near the side walls were trapped.

The wavelength of the sinusoidal microelectrode affects trapping efficiency. The shorter the wavelength, the higher the trapping efficiency (data not shown). When the wavelength of the microelectrode is shortened, the gap region becomes long, causing a large capacitance.

III. EXPERIMENTS

In this study, several kinds of bacteria were tested: *Staphylococcus aureus* (NBRC 13276) (SA), *Bacillus subtilis* (NBRC 3134) (BS), and *Pseudomonas aeruginosa* (NBRC 13275) (PA). The targets are listed in Table 1. SA was cultured in liquid Nutrient Broth with 0.5% NaCl. BS and PA were cultured in liquid Luria-Bertani (LB) broth. All bacteria were incubated at 35˚C overnight. After harvesting the bacteria, they were re-suspended in 0.1 M mannitol. The number of bacteria was estimated by a colony-counting method.

Table 1. Bacteria used in this study.

Name	Strain	Typical size
Staphylococcus aureus (SA)	NBRC 13276	0.5–1.5 μm in diameter
Bacillus subtilis (BS)	NBRC 3134	2–6 μm ×1 μm
Pseudomonas aeruginosa (PA)	NBRC 13275	0.5–0.8 μm × 1.5–3 μm

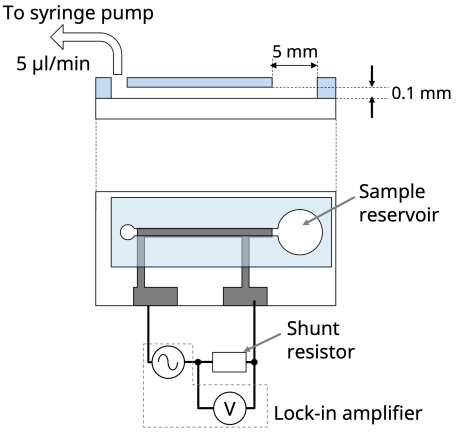

Fig. 3. Setup of the microelectrode and the microchannel.

A chromium serpentine microelectrode was fabricated on a glass substrate (Fig. 1 (b)). The wavelength and the magnitude of the gap sinusoidal wave were 0.5 mm and 0.2 mm, respectively. A polydimethylsiloxane (PDMS) microchannel whose dimension was 17 [mm] (L) × 0.6 [mm] (W) × 0.1 [mm] (H) ≈ 1 [µl] was treated by a plasma and attached to the substrate. The microchannel had an inlet reservoir and an outlet hole. A silicone tube, whose inner diameter was 1 mm, was connected to the outlet hole. The other end of the silicone tube was connected to a syringe pump. The setup of the microelectrode and the microchannel is depicted in Fig. 3. The surface of the microchannel and the microelectrode was treated by a blocking reagent before testing the bacteria suspension. The electrical circuit to carry out DEPIM consisted of the microelectrode, a shunt resistance (1 kΩ), and a lock-in amplifier. They were connected in serial [3].

The bacteria suspension of 40 µl was poured into the inlet reservoir, and then the syringe pump generated a flow of 5 µl/min. During the flow, the sinusoidal voltage of 4 V_{PP} and 100 kHz was applied to trap the target by positive DEP and measure the impedance, meaning DEPIM. The lock-in amplifier generated the voltage and measured the voltage drop at the shunt resistance. The impedance of the microelectrode was calculated using a homemade LabView program in real time.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

Fig. 4 shows DEPIM results using the interdigitated and the serpentine microelectrodes for detecting SA. At first, the microchannel, which was placed on the microelectrode, was filled with the blocking reagent. Then, the microchannel was washed with deionized water. After washing, the sample solution, SA in deionized water, was applied to the sample reservoir to avoid forming air bubbles in the microchannel. The fluid velocity was adjusted by the syringe pump connected to the microchannel outlet. DEPIM was carried out for 1 min.

When the serpentine microelectrodes were used, the DEPIM curve rose more steeply than the case of the interdigitated one. It suggests that the trapping efficiency of the serpentine microelectrodes was higher than that of the interdigitated one.

Fig. 5 shows DEPIM results detecting SA, BS, and PA using the serpentine microelectrode. The results clearly show that three kinds

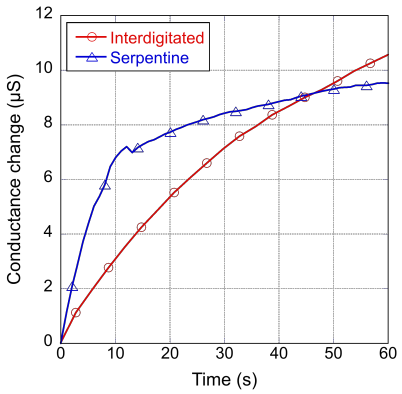

Fig. 4. Comparison between the interdigitated and the serpentine microelectrodes in detecting SA.

of bacteria were detected at the concentration of 10 cells/µl in 1 min. The concentration corresponds to 10^4 cells/ml. The typical DEPIM detection limit was demonstrated as 10^5 cells/ml. When EPA-DEPIM was utilized, the detection limit became 10^2 cells/ml. It seems that the sensitivity of the serpentine microelectrode DEPIM was ten times higher than DEPIM using interdigitated microelectrode and was worse than EPA-DEPIM.

The DEPIM curve shown in Fig.4 rose more steeply than that shown in Fig. 5 (a). This was because of the concentration. The high-concentration solution, about 10^6–10^7 cells/µl, emphasized the effect of the uses of the serpentine microelectrode.

Typical DEPIM requires circulating the sample solution for at least 10 min to detect 10^5 cells/ml. Therefore, it requires several ml of the sample solution. On the other hand, to detect 10^2 cells/ml, EPA-DEPIM requires the DEP trapping process by circulating the sample solution for at least three hours before applying the EPA signal. In this study, DEPIM using serpentine microelectrode represents 10 cells/µl in 1 min, corresponding to 50 cells. If the typical DEPIM uses 5 ml of 10^5 cells/ml, this corresponds to 5×10^5 cells. Regarding the number of cells, the sensitivity of the serpentine microelectrode

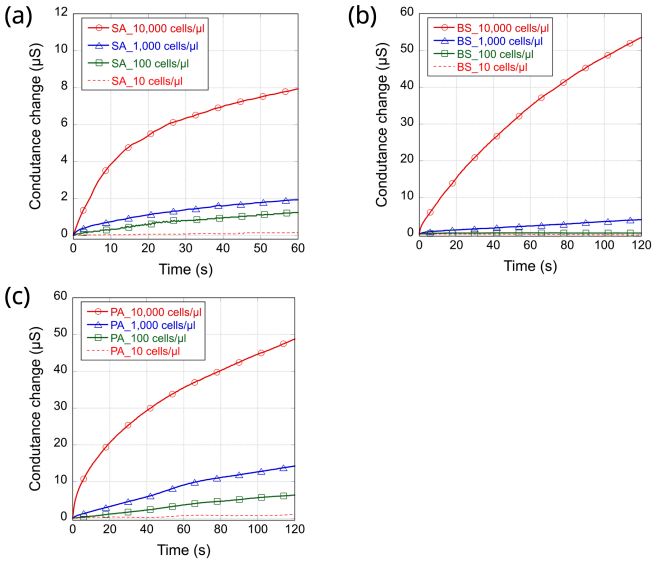

Fig. 5. DEPIM curves to detect SA (a), BS (b), and PA (c) in the suspension with the variation of the concentration.

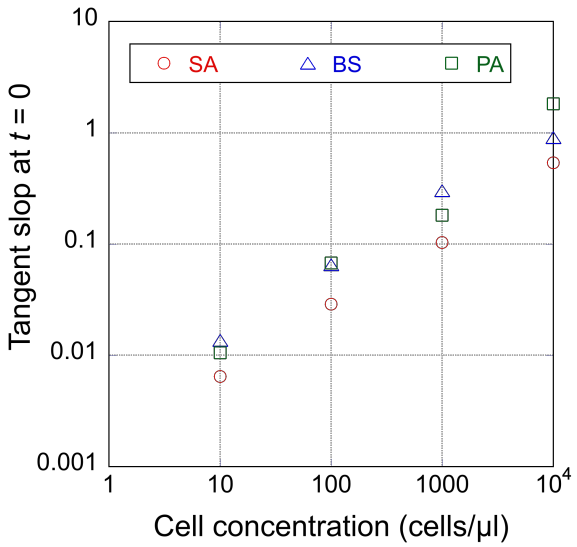

Fig. 6. Plot of the tangent slopes of DEPIM curves as a function of the cell concentration.

increased 10^4 times higher than that of the interdigitated microelectrode. We believe the serpentine microelectrode is suitable for testing a small amount of the sample solution.

DEPIM curves are quantified by determining the tangent slope of the curves at $t = 0$, where t is time. Fig. 6 shows the tangent slopes of each DEPIM curve. Tested three kinds of bacteria represented similar detection profiles. However, SA showed a slightly lower sensitivity than the other two bacterium types. The difference in the sensitivity could be derived from the size of the bacteria. As shown in Table 1, BS and PA are larger than 1 μm and are rod-like in shape, while SA is almost 1 μm and sphere-like. This size and shape difference suggests that more SA is required to make measurable conductance change when positive DEP traps it between the 5 μm gap rather than in the case of BS and PA.

Several kinds of microelectrodes have been proposed [7]. To the best of the author's knowledge, the microelectrode having a sinusoidal waveform serpentine gap region has never been reported elsewhere. The electrode covers the entire microchannel width with a single gap region to capture target bacteria in laminar flow by DEP. In contrast, the interdigitated microelectrode needs to increase the number of gap regions. The other waveform-like gap designs, such as square and triangle (saw-tooth) waveforms, can be considered. Such waveforms are not smooth and have acute angles at the bending points. At the points, the intense electric field forms rather than the remaining gap region. The intense electric field points will trap more bacteria and make a stack of the trapped bacteria. The stacking could decrease the sensitivity of DEPIM because the stacked bacteria do not contribute to the change of the microelectrode impedance. The sinusoidal waveform gap makes the electric field distribution in the gap the same during the entire gap region. Therefore, the proposed microelectrode can effectively capture target bacteria by DEP without increasing the impedance, meaning it is better for DEPIM.

V. CONCLUSION

In this study, a serpentine microelectrode having a sinusoidal waveform gap was proposed to improve the sensitivity of DEPIM. The new microelectrode design was compared with the conventional interdigitated microelectrode in terms of numerical simulation and experiments. The numerical simulation shows that when the serpentine microelectrode is used, all the targets in a laminar flow in a microchannel are trapped, whereas the interdigitated ones are not. The experiments to detect three kinds of bacteria demonstrated that the serpentine microelectrode represented a sensitivity 10^4 times higher than the interdigitated microelectrode. The new microelectrode design is suitable for detecting bacteria in small amounts of the test solution.

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