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Characterization of 20 µm Dielectrophoretic Interelectrode Gap for Staphylococcus Aureus Rapid Detection Application

Arash Zulkarnain Ahmad Rozaini[a,b], Abdullah Abdulhameed[c], Revathy Deivasigamani[a], Noraziah Mohamad Zin[b], Amin Ahmad Kayani[d], Burhanuddin Yeop Majlis[a] , and Muhamad Ramdzan Buyong[a,*]

[a]*Institute of Microengineering and Nanoelectronics (IMEN), Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia.*
[b]*Center for Diagnostic, Therapeutic and Investigative Studies Faculty of Health Sciences Universiti Kebangsaan Malaysia Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.*
[c]*Center for Communication Systems and Sensing, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia.*
[d]*School of Engineering, RMIT University Melbourne, Australia.*
**Corresponding author. e-mail: muhdramdzan@ukm.edu.my*

ABSTRACT

We propose an improvement of dielectrophoresis technique (DEP) by designing, simulating and experiment a 20 µm interelectrode gap for Staphylococcus aureus rapid detection application. In this paper, we use MyDEP simulation for DEP polarization Staphylococcus aureus on frequencies range. COMSOL simulation is utilized for comparison of 20 µm and 80 µm interelectrode gap based on trajectory and velocity of particles for Staphylococcus aureus application. We implied 20 µm interelectrode gap for Staphylococcus aureus application produced higher magnitude DEP force. Which gives accurate trajectory characterization and higher velocity of particle movement. DEP characterization using small interelectrode gap is capable of producing stable and optimum DEP value. It is demanding to distinguish the simulation Staphylococcus aureus using experimental method. DEP technique is important for analysis and characterization of the bacteria. The result show that 20 µm interelectrode gap has higher intensity of electric field which is 1.51 x 10^6 V/m in COMSOL simulation and produced 20.1 m/s for velocity of particle trajectories which is higher compared to 80 µm interelectrode gap. The DEP response was tested on 2.5 MHz, as crossover frequency response was observed on experimental and simulation. Thus, supportsthe capability of 20 µm interelectrode gap for Staphylococcus aureus rapid detection application. Furthermore, DEP characterization can be improvised by focusing on interelectrode gap for characterization among bacteria cells, critical for bacteria detection, manipulation, and isolation.

Keywords: *Dielectrophoresis, Staphylococcus aureus, interelectrode gap, frequency, detection*

1. INTRODUCTION

Over 1.5 million deaths were caused by bacterial infection from a multi-drug resistant bacterium especially Staphylococcus aureus [1,2]. Dielectrophoresis (DEP) technique is a critical approach on microbial characterization for solving the bacterial infection. Characterization of Staphylococcus aureus using DEP for DEP response pDEP, crossover frequency and nDEP in this recent years is important for detection, manipulation and isolation of the cells [3,4]. Based on past research, DEP devices focusing on 80 µm interelectrode gap for biological application [5–9]. In this work, we improvised DEP technique by decreasing interelectrode gap device to 20 µm for characterization of Staphylococcus aureus. We demonstrated the simulation of dielectrophoretic behavior and velocity of particle movement between two different gap of microelectrode which are 80 µm and 20 µm using COMSOL. This study has provided an enhance validation of 20 µm interelectrode gap use for the real-time DEP for characterization of Staphylococcus Aureus bacteria.

2. DEP THEORY

DEP is the motion of particles in a non-uniform electric field that utilized for the particle's polarization[10,11]. DEP polarization is determined by the physical properties of the particles and the medium, such as permittivity and conductivity.

$$\mathbf{F_{DEP}} = 2\pi r_{ext}^3 \varepsilon_0 \varepsilon_{rms}\, \mathrm{Re}[f_{CM}] \nabla E^2 \quad (1)$$

Consequently, the connection of dynamic dielectric properties of particles, medium, and electric field are abbreviated using the Clausius Mossoti- Factor (CMF) in DEP force F_{DEP} formulation as in Equation 1, where ε_m is the medium permittivity r_{ext} is the particle radius, and $Re[f_{CM}]$ is the real part CMF of the particle, and ∇E^2 defines the gradient of the external field magnitude square. $Re[f_{CM}]$ is the Clausius-Mossotti factor of the particle in a medium and for a spherical particle. ε_0 is the permittivity for vacuum, 8.854 × 10-12 F/m. CMF of particle are based of dynamic dielectric properties of the particle with the surrounding medium. CMF can have a value between 1 and -0.5 which consist of pDEP, nDEP and crossover frequency (fxo). CMF of positive values indicated for positive DEP (pDEP), and the particles attracted to higher electric field regions. The CMF of negative values showed negative DEP

(nDEP), and the particle repulsed from higher electric field regions. At the value of 0, the pDEP and nDEP forces vanish and showed this static condition is known as the crossover frequency (fxo). The manipulation of these response can be applied for manipulation, separation and characterization of particles in medium especially biological particles.

3. MATERIALS AND METHOD

The electrical field simulation of Staphylococcus aureus has been done using MyDEP and COMSOL Multiphysics. MyDEP simulation used to investigate the dielectric response of bacteria particles suspended in a medium under AC electric fields [10]. COMSOL software was used as a second step to predict trajectories and velocity in a static droplet representing zero initial velocity using non-uniform electric field.

In this work, the MyDEP tool was utilized to analyze the frequency response of the bacteria to plot the CMF curve in medium. Since the bacteria is a complex spherical particle, a double shell model with a cell wall, cell membrane and cytoplasm were considered [12–16]. Thus, the 'bacteria's physical and electrical properties will contribute in calculating its equivalent relative permittivity and electrical conductivity[17].

By using 80 µm and 20 µm interelectrode gap, the trajectories and velocity of bacteria simulated based on the electric field and DEP magnitude force using COMSOL. Different in interelectrode gap and controlling the bacteria's physical and electrical properties will contribute to calculating its equivalent relative permittivity and electrical conductivity respectively to its gap [4,18]. The equivalent conductivity and permittivity of the bacteria in a medium will determine the particle trajectories, velocity and electric field.

3.1. Parameter

The bacteria's equivalent relative permittivity and conductivity and the frequency range were tabulated in table 1 and included in the MyDEP and COMSOL simulation (COMSOL Inc., Burlington, MA, USA) [7,10,11]. MyDEP and COMSOL was used to solve the FDEP exerted on the bacteria placed in the distilled water at different frequencies.

Table 1 Staphylococcus aureus properties

Specifications	Staphylococcus aureus
Diameter of bacteria	1.0 µm [19]
Shell thickness Th_{cw}	30 nm [15]
Cell wall conductivity σ_{cw}	6×10^{-3} [18]
Cell wall relative permittivity ε_{cw}	60 [6]
Cell membrane thickness Th_{cm}	8 nm[15]
Cell membrane conductivity σ_{cm}	2.5×10^{-8} S/m [4]
Cell membrane relative permittivity ε_{cm}	6 [4]
Cytoplasm conductivity σ_{cv}	7.5×10^{-5} S/m [4]
Cytoplasm relative permittivity ε_{cv}	60 [4]

3.2. Geometry of Microelectrode

The 80 µm and 20 µm interelectrode gap were designed following Figure 1 where the study was conducted based on the design to obtain the electric field distribution, particle trajectories and velocity on two different gaps.

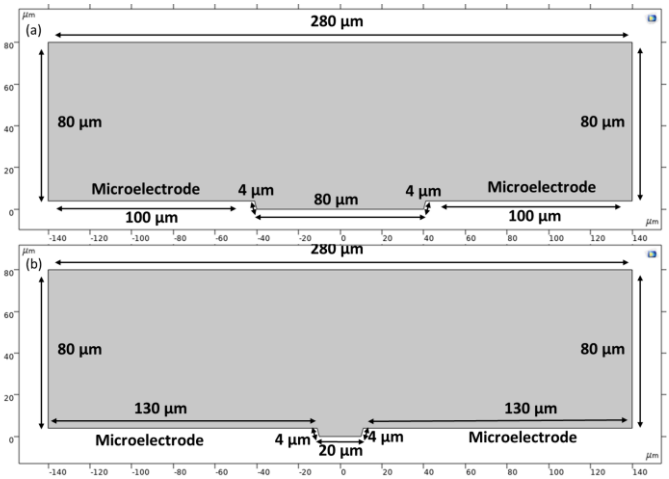

Figure 1. dimensions of the simulation geometry (a) 80 µm interelectrode gap (b) 20 µm interelectrode gap.

3.3. Study Solution and Mesh in COMSOL

A stationary study followed by a frequency domain study was conducted to obtain the electric field distribution of the interelectrode gap. Time dependent study is modelled in this simulation to observe the particle trajectories and velocity. The medium and particles was designed as a static droplet with scattered particles covered with a glass slide with no inlet and outlet condition [7]. Figure 2 shows the meshing simulation geometry of 80 µm and 20 µm interelectrode gap.

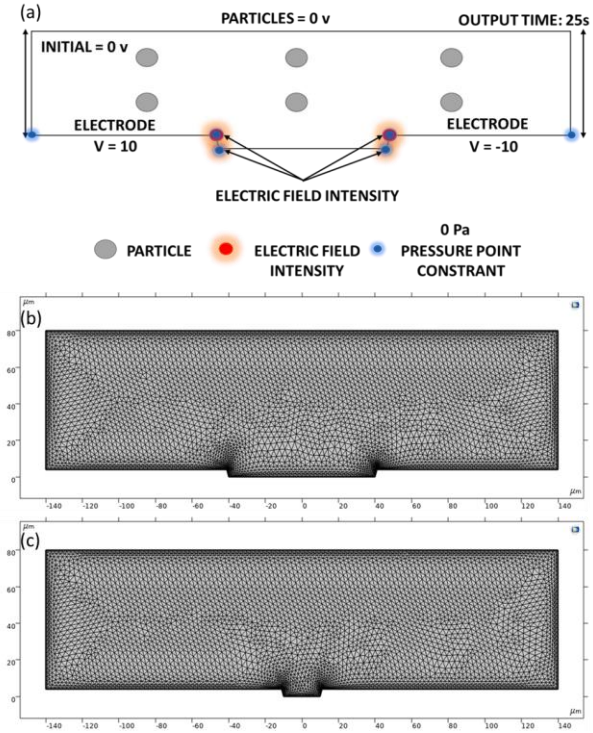

Figure 2. Side view: (a) Boundary condition of simulation and mesh of simulation geometry of (b) 80 µm interelectrode gap (b) 20 µm interelectrode gap.

3.4. Fabrication

The manipulation of particle is visualized under microscope to observe the trajectory of the particle when applied with DEP [5,20]. The electrode is fabricated using lift off process. The interelectrode gap is 20 µm to allow the bacteria visualization and characterization under microscope.

3.5. Experimental Setup

The electrodes are placed under a microscope OLYMPUS-BX53M as shown in Figure 3 and directly connected to an AC function generator WaveStation Function 2022 25 MHz /Arbitrary Waveform Generator through a prober. The function generator is set to supply an AC signal of 6 V peak to peak. The signal amplitude selection is based on the medium conductivity, while the frequency was based on the MyDEP model simulation results according to the input parameter. The bacterial manipulation is monitored from the microscope by Olympus cell Sens standard software from top view towards the microelectrode. The region of interest (ROI) of microelectrode is between the 20 µm gap for movement of particles when applied with DEP.

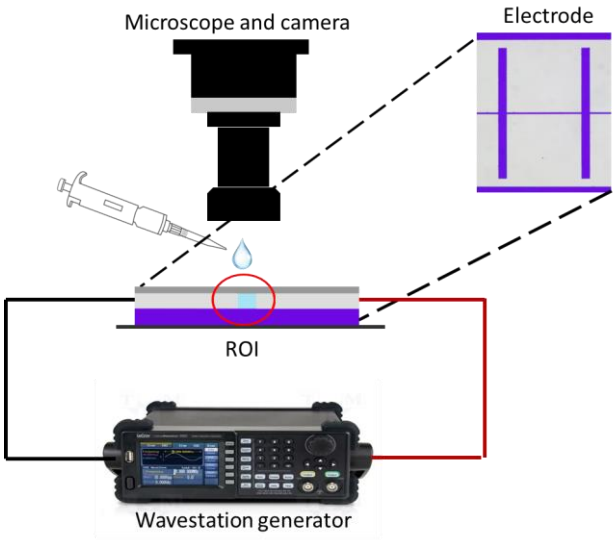

Figure 3. DEP experimental set up.

4. RESULT AND DISCUSSION

4.1. MyDEP Simulation

The simulation result of the CMF polarization is shown Figure 4 of Staphylococcus aureus shows pDEP, nDEP and crossover frequency on distilled water medium.

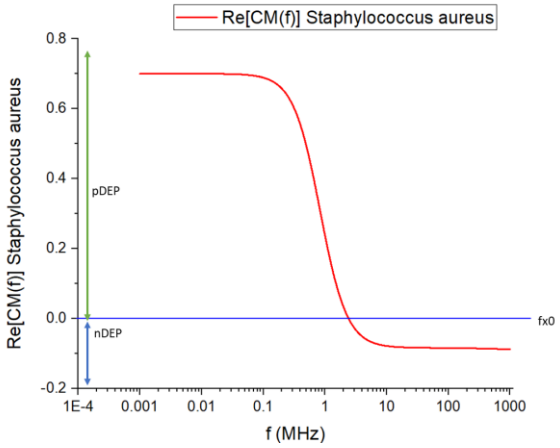

Figure 4. MyDEP simulation: Model polarization factor efficiency of CMF Staphylococcus aureus (red line), crossover frequency at 2.5 MHz for Staphylococcus aureus.

The result shows nDEP, pDEP and fx0 on distilled water (σ= 0 S/m). The properties of Staphylococcus aureus interpreted into electrical properties thus plot the CMF polarization. The polarization of cell wall Staphylococcus aureus begins with pDEP [18]. At frequencies beyond the pDEP level to gradually fall, become nDEP. The fxo shows 2.5 MHz for Staphylococcus aureus when the red line reach zero which static movement for bacteria.

4.2. COMSOL Simulation

Figure 5 shown the electric field distribution of 20 µm interelectrode gap microelectrodes is calculated. The highest intensity field on COMSOL observed is 1.51 x 10^6 V/m while on 80 µm interelectrode gap, the intensity of electric field is on 6.06 x 10^5 V/m. The simulation result indicates that on smaller gap microelectrode, the electric field highest can be exploited from the design thus can increase the magnitude force in DEP in manipulation and characterization of bacteria particles.

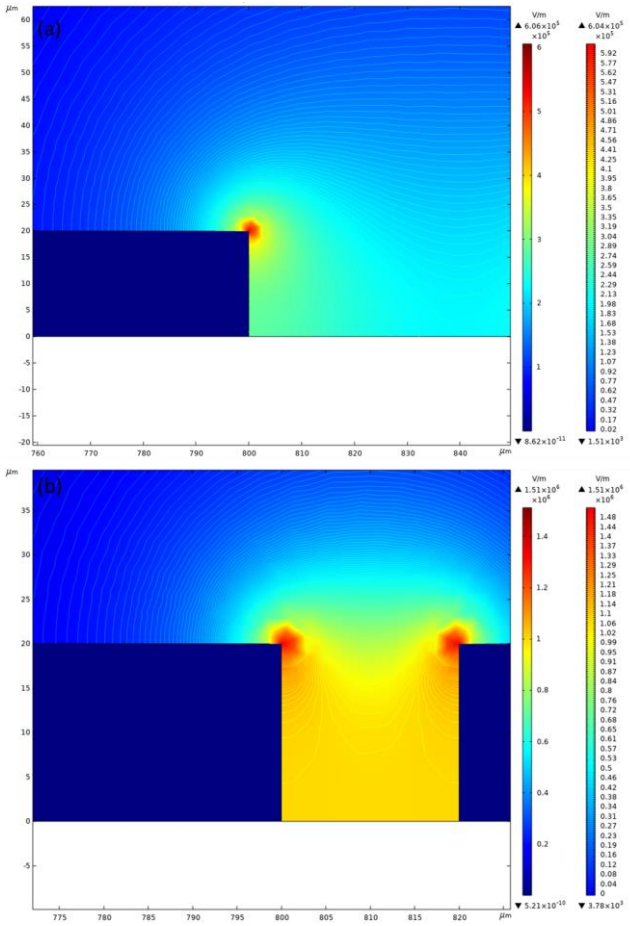

Figure 5. COMSOL simulation (Side view): Electric field intensity of (a) 80 µm interelectrode gap 6.06 x 10^5 V/m (b) 20 µm interelectrode gap 1.51 x 10^6 V/m.

This is in line with Coulomb's law in physics which describes the relationship between the electric force between two charged objects and the electric field. The resistance between two-point charges also decreases at low spacing. This gives a factor to increase the electric field strength as shown in the COMSOL simulation [21].

Next, the bacteria trajectories and velocity of particles when applied with DEP in medium is simulated on both microelectrodes. It is demanding to distinguish the simulation of characterization of Staphylococcus aureus using COMSOL on two different microelectrodes by its trajectories and its velocity. The initial position of the particles in Figure 6 (a) and Figure 7 (a) represents bacteria cells at 0s (before applying the electric field). The results of Figure 6 and Figure 7 are demonstrated to be simulation of Staphylococcus aureus on 80 µm interelectrode gap and 20 µm interelectrode gap on different frequency ranges for 25s.

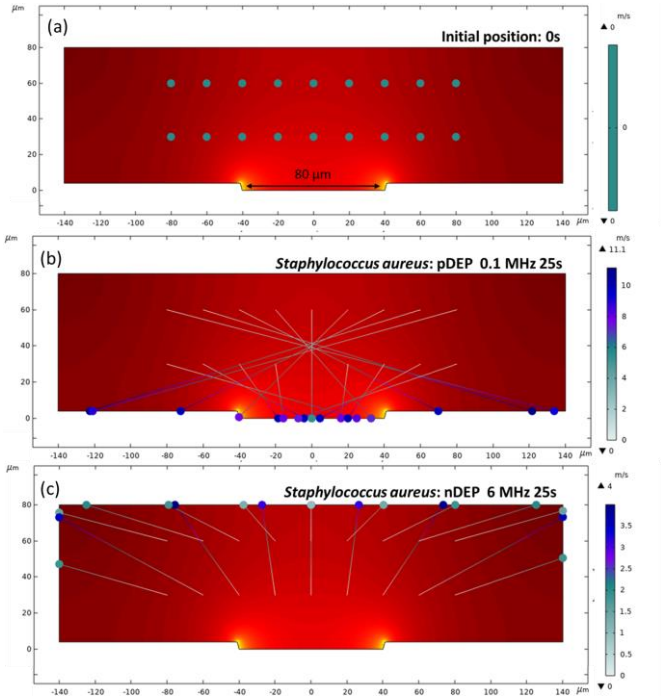

Figure 6. COMSOL particle trajectory and velocity simulation 80 µm interelectrode gap: (a) initial position at 0 s with velocity of 0 V/m (b) pDEP: bacteria attracted towards high electric field at 0.1 MHz with velocity of 11.1 m/s (c) nDEP: bacteria repelled away from high electric field at 6 MHz with velocity of 4 m/s.

Figure 6 (b) shows the particle trajectory representing Staphylococcus aureus moving towards high electric field intensity regions on interelectrode gap 80 µm. Corresponding to pDEP at a frequency of 0.1 MHz. The motion of bacteria cell reaches regions near the electrode edges and electrode gap on velocity of 11.1 m/s. On 6 MHz, Staphylococcus aureus moved towards a lower electric field which reflects nDEP with velocity of 4 m/s as shown in Figure 6(c).

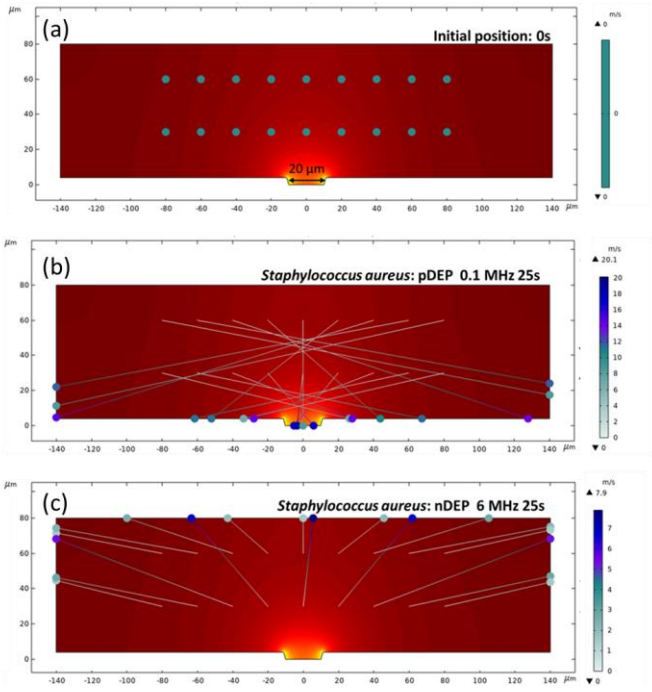

Figure 7. COMSOL particle trajectory and velocity simulation 20 µm interelectrode gap: (a) initial position at 0 s with 0 m/s velocity (b) pDEP: bacteria attracted towards high electric field at 0.1 MHz with velocity of 20.1 m/s (c) nDEP: bacteria repelled away from high electric field(6 MHz) with velocity of 7.9 m/s.

Figure 7 shows particle trajectories and velocity of Staphylococcus aureus on interelectrode gap 20 µm. While the trajectory of particles is similar on pDEP and nDEP with 80 µm interelectrode gap, the velocity of the particles increases. pDEP on interelectrode gap 20 µm shows 20.1 m/s which is higher compared to 80 µm interelectrode gap. On nDEP response, the velocity increase to 7.9 m/s. Table 2 shows the Intensity of electric field and velocity of DEP response on 80 µm and 20 µm interelectrode gap.

Table 2 Intensity of electric field and velocity of DEP response on 80 µm and 20 µm interelectrode gap

Interelectrode gap	Intensity of electric field V/m	Velocity of pDEP response	Velocity of nDEP response
80 µm	6.06 x 10^5 V/m	11.1 m/s	4 m/s
20 µm	1.51 x 10^6 V/m	20.1 m/s	7.9 m/s

Simulation of the bacteria suspended within the microelectrode show a uniform distribution of bacteria cells with higher electric field power thus giving much better DEP force on 20 µm. The bacteria with smaller size in micrometer which gives us an insight to the dielectric properties of bacteria Staphylococcus aureus can be observed better on smaller gap microelectrode. Which produce higher magnitude DEP thus gives accurate characterization DEP.

4.3. DEP Experimental

The experimental DEP is tested to confirm the integrity of 20 µm interelectrode gap for DEP characterization. The crossover frequency in MyDEP which is 2.5 MHz for isolation of Staphylococcus aureus, which could lead to deeper levels of analysis on specific factors. The result can be utilized as an indication for crossover frequency characterization and isolation for Staphylococcus aureus. On this response, no bacteria movement were detected thus induced crossover frequency response.

Table 3 DEP experimental and MyDEP simulation of Staphylococcus aureus

DEP response	MyDEP	Dep experimental
pDEP	<2.5 MHz	<2.5 MHz
Crossover frequency	2.5 MHz	2.5 MHz
nDEP	>2.5 Mhz	>2.5 Mhz

The results of Figure 8 are demonstrated to be highly predictive for whole DEP characterization, the crossover frequency is potentially applicable. The experimental result of microscopic image on Figure 8 shows constant movement of particle which indicate fxo on 2.5 MHz for Staphylococcus aureus.

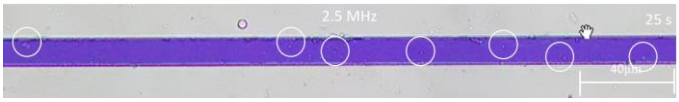

Figure 8. DEP experimental: No movement of bacteria particles detected for 25 s on 2.5 MHz.

5. CONCLUSION

These differences of microelectrode may arise in various parts in the gap of microelectrode between 80 µm and 20 µm are likely to cause effect in polarization of DEP response due to the electric field. The differentiating and characterizing bacteria based on utilized by physical properties will be better view on 20 µm microelectrode gap with higher magnitude of DEP. The DEP crossover frequencies is a stable technique in characterizing Staphylococcus aureus bacteria with reliable baseline and validified by the conventional method, promising a rapid, accurate and scalable methodology.

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