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Akmal Suhaimi
National University of Malaysia
Muhammad Faez Aris
National University of Malaysia
Arash Zulkarnain
National University of Malaysia
Noraziah Mohamad Zin
National University of Malaysia
Muhamad Ramdzan Buyong
muhdramdzan@ukm.edu.my
National University of Malaysia

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Voltage-Dependent Dielectrophoretic Response of Bacterial Cells: Implications for Reliable Cell Manipulation

Akmal Suhaimi[1], Muhammad Faez Aris[2†], Arash Zulkarnain[1†], Noraziah Mohamad Zin[2*], Muhamad Ramdzan Buyong[1*]

[1]Institute of Microengineering and Nanoelectronics (IMEN), Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia.
[2]Centre for Diagnostic, Therapeutic and Investigative Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, 50300, Malaysia.

*Corresponding author(s). E-mail(s): noraziah.zin@ukm.edu.my; muhdramdzan@ukm.edu.my;
Contributing authors: p137669@siswa.ukm.edu.my; muhammadfaez848@gmail.com; arashzulkarnain@gmail.com;
†These authors contributed equally to this work.

Abstract

Rapid bacterial detection is important for environmental monitoring, particularly for identifying microbial contamination in water resources. Dielectrophoresis (DEP) provides a label-free approach for bacterial detection by exploiting differences in cellular dielectric properties. Our previous studies established bacterial movement velocity, force, and crossover frequency as quantitative parameters toward the development of a DEP-based detection platform. However, optimization of the operating conditions is necessary to ensure reliable measurement. During voltage optimization, unexpected positive DEP (pDEP) was observed at low frequencies where negative DEP (nDEP) was expected, particularly at 20 Vpp. This study therefore investigated whether applied voltage affects bacterial viability and membrane integrity during DEP experiment. *Escherichia coli* and *Staphylococcus aureus* (pH 7), were exposed to 3–20 Vpp at 50 kHz, followed by pDEP quantification, colony-forming unit (CFU) analysis, Live/Dead fluorescence staining, and transmission electron microscopy (TEM). Despite increasing pDEP occurrence at higher voltages, CFU analysis showed no significant differences in culturability (p ¿ 0.05). In contrast, fluorescence and TEM analyses

revealed voltage-dependent changes in membrane integrity and structure. These findings indicate that DEP exposure can induce sublethal membrane perturbation sufficient to alter bacterial dielectric response without measurable loss of culturability. Appropriate voltage selection is therefore critical for preserving measurement reliability in DEP-based bacterial detection and its future application to environmental monitoring.

Keywords: Dielectrophoresis, Cell Membrane Integrity, Cell Viability, Voltage

1 Introduction

Pathogenic bacteria can enter and persist in freshwater environments, including rivers, lakes, reservoirs, and groundwater, posing potential risks to environmental quality and human health Pandey et al. (2014). Consequently, reliable and timely detection of bacterial contamination is essential for effective environmental monitoring and water-quality assessment. Conventional culture-based methods remain widely established for bacterial detection and identification owing to their reliability and broad applicability Zukowska (2021); Ferone et al. (2020). Nevertheless, these approaches are labor-intensive and time-consuming, with definitive identification potentially requiring several days Ferone et al. (2020). These limitations have driven the development of rapid and alternative bacterial detection technologies, among which Dielectrophoresis (DEP) has emerged as a promising approach Petrovszki et al. (2021); Mohd Maidin et al. (2021); Kumar et al. (2020). DEP enables the manipulation of bacterial cells through their interaction with a non-uniform alternating electric field and has been explored for the detection, separation, and concentration of bacteria, including those present in contaminated water. Importantly, its frequency-dependent and label-free response to the intrinsic dielectric properties of bacterial cells provides a basis for developing rapid platforms for bacterial detection and characterization.

Our previous studies have focused on developing this DEP mechanism into a quantitative bacterial detection platform Suhaimi et al. (2025); Zulkarnain et al. (2026). The next stage of this approach requires optimization of the operating conditions of the DEP system. Frequency, medium conductivity, and applied voltage are particularly important because they determine the DEP response and the movement of bacterial cells Pethig (2013). Applied voltage is especially relevant to velocity-based analysis, as sufficient DEP force is required to generate measurable bacterial displacement. Therefore, an appropriate operating voltage must provide sufficient movement for quantitative analysis while maintaining a reliable representation of the bacterial response.

During optimization of the DEP operating conditions, an unexpected shift in bacterial DEP behavior was observed. Under the low-conductivity conditions employed in our DEP system, both *E. coli* and *S. aureus* typically exhibited negative DEP (nDEP) within the low-frequency range of approximately 10–100 kHz. However, as the applied voltage was increased to 20 Vpp, an increasing proportion of bacterial cells migrated toward the electrodes, indicating a transition to positive DEP (pDEP) within the

same frequency range. The increase in DEP force with increasing applied voltage is well established and was therefore not the concern of this observation. Instead, the unexpected change from nDEP to pDEP raised a more important question regarding whether the higher operating voltage was affecting the bacterial cells themselves. If the conditions used to generate a stronger measurable DEP response simultaneously alter the biological or dielectric state of the target, the resulting response may no longer accurately represent the original properties of the bacteria. This presents a potential limitation for a DEP-based detection platform, particularly when bacterial dielectric behavior is used as the parameter for characterization.

Therefore, this study investigates whether applied voltage during DEP exposure affects bacterial viability and membrane integrity and whether these effects are associated with the unexpected DEP behavior observed at higher voltage. A fixed frequency of 50 kHz was selected within the observed nDEP region, while the applied voltage was systematically varied from 3 to 20 Vpp using *E. coli* and *S. aureus* as representative Gram-negative and Gram-positive bacteria. The occurrence of pDEP was quantified at each voltage, followed by recollection of the DEP-treated cells for colony-forming unit (CFU) analysis to determine their ability to remain culturable. Live/Dead fluorescence staining was used to assess changes in membrane integrity, while transmission electron microscopy (TEM) was used to examine the bacterial cell envelope following DEP exposure. Through these complementary analyses, this study aims to determine whether the operating voltage used for DEP measurement can influence the condition of the bacterial target and to establish an appropriate operating range for reliable DEP analysis. Addressing this limitation is an important step in the continued development of the DEP platform toward rapid bacterial detection in environmental samples.

2 Results

The DEP behavior of bacterial cells was first evaluated at 50 kHz which corresponds to nDEP. Under this condition, cells are expected to move away from the electrode gap. However, as the applied voltage increased, an unexpected behavior was observed. Several cells began to move toward the electrode edge, indicating pDEP response. Thus the number of pDEP bacterial cells was counted.

Based on Figure 1a below, for *E. coli*, no pDEP response was observed at low voltages (3-5 Vpp). At 10 Vpp, a small number of cells exhibited pDEP (31 $\pm$ 9.17 cells), and this increased further at 15 Vpp (82 $\pm$ 19.52 cells) and 20 Vpp (315 $\pm$ 23.43 cells). Statistical analysis showed no significant difference between low-voltage conditions (3-10 Vpp), while higher voltages ($\geq$ 15 Vpp) resulted in a significant increase in pDEP response (p ¡ 0.05). In contrast, *S. aureus* showed negligible pDEP response from 3 - 15 Vpp, with a noticeable increase only at 20 Vpp (11 $\pm$ 4.58 cells), which was significantly different from all lower voltages.

These results demonstrate that increasing voltage alters DEP behavior in a voltage-dependent and species-dependent manner. The effect is clearly more pronounced in *E. coli*, while *S. aureus* remains relatively stable across most conditions.

An initial hypothesis was that the observed change in DEP behavior could be due to cell death at higher voltages. If the cells were no longer viable, their electrical

properties could change and affect their response to the electrical field. To evaluate this, CFU analysis was performed.

The CFU results showed no statistically significant differences (p ¿ 0.05) across all voltage conditions for both *E. coli* and *S. aureus*. Although *E. coli* exhibited a slight decreasing trend at higher voltages, the cells remained viable. Similarly, *S. aureus* showed minimal variation across all conditions. CFU analysis can be found in table 1 and figure 1b below.

Table 1 CFU count of every sample recollected after DEP exposure

Voltage	*E. coli*	*S. aureus*
Control	1.34E07 ± 1.44E06	1.51E07 ± 7.54E05
3 Vpp	1.25E07 ± 9.29e06	1.43E07 ± 4.04E05
5 Vpp	1.15E07 ± 1.00E05	1.41E07 ± 2.51E05
10 Vpp	1.11E07 ± 3.21E05	1.40E07 ± 1.73E05
15 Vpp	1.13E07 ± 5.56E05	1.41E07 ± 3.60E05
20 Vpp	8.67E06 ± 1.36E05	1.41E07 ± 7.37E05

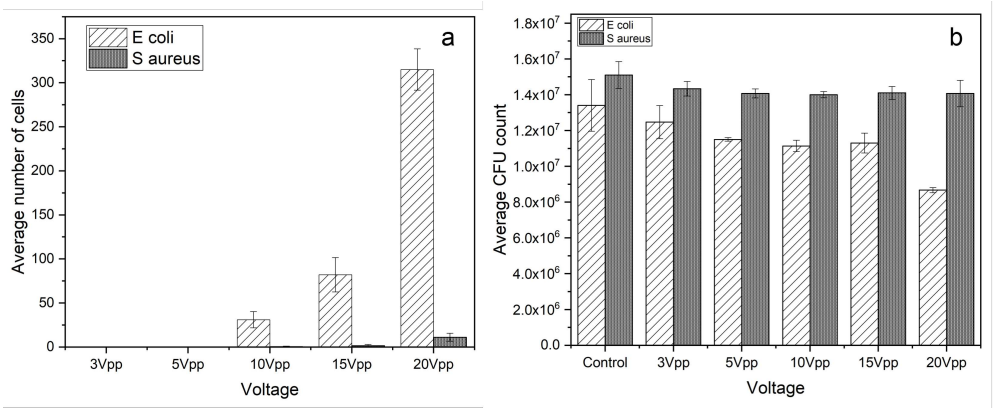


Fig. 1 Figure 1(a) shows the number of *E. coli* and *S. aureus* cells that were trapped at the edge of the electrode (pDEP) in 50 kHz frequency, while Figure 1(b) shows the number of colony formed on nutrient agar for both bacteria that grew from the recollected sample from the electrode after each DEP exposure

These findings confirm that the observed DEP behavior is not caused by complete cell death. The cells remain viable even at higher voltages, suggesting that the initial hypothesis is to be dismissed that high voltages in DEP do not cause any bacterial death.

To further investigate this, membrane integrity was assessed using Live/Dead fluorescence staining. For *E. coli*, all cells remained intact at low voltages (3-5 Vpp). At higher voltages, a clear increase in membrane-compromised cells was observed, with 18 ± 3.61% at 10 Vpp, increasing to 49.67 ± 5.03% at 15 Vpp and 65 ± 4.58% at 20 Vpp. For *S. aureus*, no membrane damage was observed up to 10 Vpp, while moderate levels of membrane-compromised cells appeared at 15 Vpp (28.67 ± 2.52% and 20 Vpp (35.67 ±3.06). Results for Live/Dead staining can be referred to figure 2.

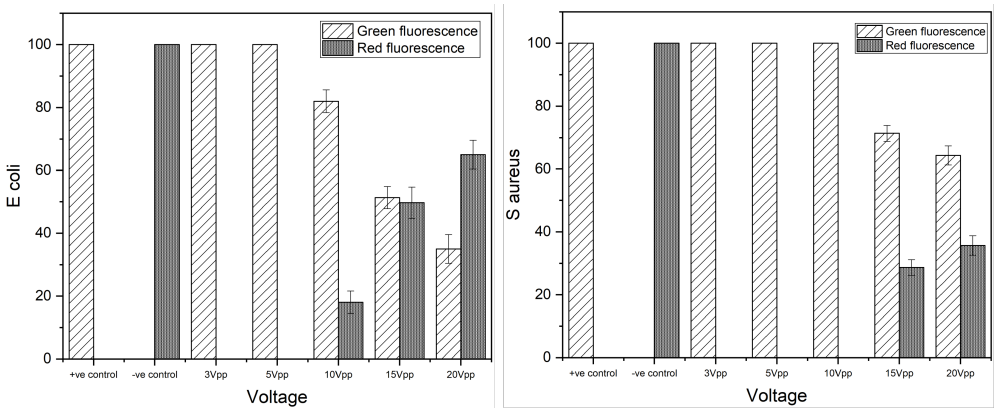


Fig. 2 The number of cells for *E. coli* and *S. aureus* with normal cell membrane (green fluorescence) and compromised cell membrane (red fluorescence)

Importantly, many membrane-compromised cells were still able to move when observed under a fluorescence microscope. This observation indicates that membrane damage does not immediately lead to loss of cellular function. Instead, the cells sustain sublethal damage that does not compromise viability but induces measurable alterations in their physical and structural properties.

This interpretation is consistent with previous studies. Zhang et al. (2023) demonstrated that electric fields can induce membrane damage in *S. aureus* while maintaining high survival rates Zhang et al. (2023). Peng et al. (2020) also reported that electric fields can induce sublethal stress, where cells temporarily lose functionality but recover after treatment Peng et al. (2020). In addition, Mahnic-Kalamiza et al. (2022) and Lu et al. (2023) described reversible electroporation, where temporary pores form in the membrane and later reseal Mahnič-Kalamiza and Miklavčič (2022); Lu et al. (2023). These mechanisms explain membrane damage without a loss of viability.

To further confirm the structural effect of voltage, TEM imaging was performed. For *E. coli*, the apparent membrane appears to be swelling, with thickness increasing significantly with voltage, from approximately 12-14 nm at low voltages to 53.70 ± 16.13 nm at 20 Vpp. For *S. aureus*, the membrane remained relatively stable at lower

voltages and showed noticeable increases only at higher voltages, reaching approximately 43-44 nm. Table and figure shows the deviation of cell membrane thickness for both bacteria across all voltage sweep.

Table 2 Cell membrane thickness (nm) measured in TEM imaging of *E. coli* and *S. aureus*

Voltage	*E. coli*	*S. aureus*
3 Vpp	12.41 ± 1.65	34.33 ± 4.34
5 Vpp	13.81 ± 2.48	31.01 ± 2.47
10 Vpp	38.29 ± 9.00	29.84 ± 3.49
15 Vpp	39.79 ± 12.93	44.50 ± 7.21
20 Vpp	53.69 ± 16.13	43.29 ± 8.68

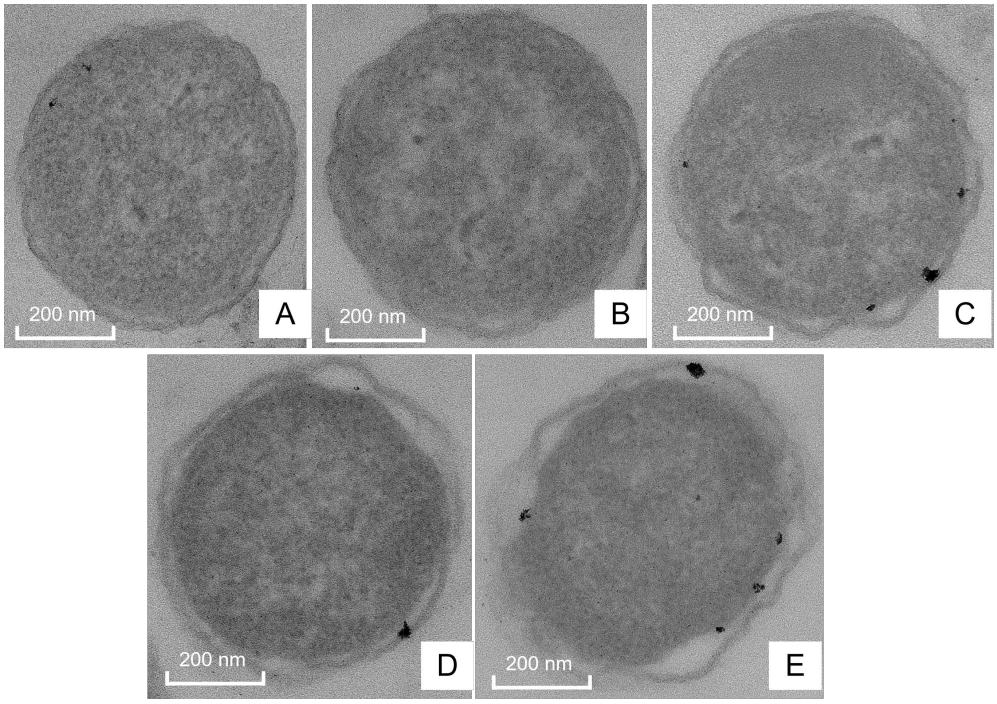


Fig. 3 TEM imaging of *E. coli* cell membrane thickness

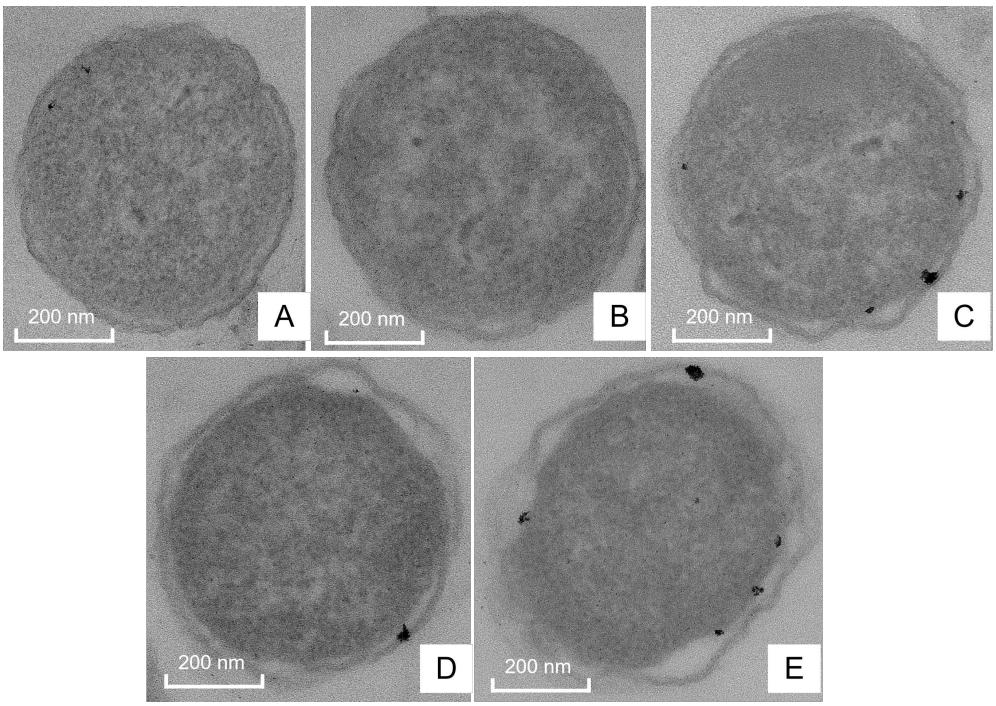


Fig. 4 TEM imaging of *S. aureus* cell membrane thickness

These results indicate that DEP exposure induces structural changes in the membrane, likely i the form of swelling or deformation. Similar observations have been reported in studies on sublethal membrane stress. Vasilchenko et al. (2019) showed that membrane-active agents can disrupt the membrane without causing immediate cell death Vasilchenko and Rogozhin (2019). Li et al. (2024) reported that *S. aureus* can enter a stress-adapted state under membrane-targeting conditions while remaining viable Li et al. (2024). Cordiso et al. (2025) and Christodoulou et al. (2022) further demonstrated that sublethal stress can trigger cellular responses without complete inactivation Christodoulou et al. (2022); Cordisco and Serra (2025).

A clear difference between *E. coli* and *S. aureus* was observed throughout this study. *E. coli* showed more pronounced membrane damage and DEP response changes, while *S. aureus* remained more resistant. This difference may be attributed to variations in cell envelope architecture and mechanical properties. Although Lee et al. (2024) reported comparable stiffness values between the two species, other studies suggest that *S. aureus* has greater resistance to mechanical stress due to its thicker cell wall Lee et al. (2024); Fitriyanti et al. (2025); Huang et al. (2023). This may lead to a threshold-dependent response, whereby structural alterations occur only when the applied stress exceeds a critical level.

Overall, these findings indicate that high-voltage DEP does not kill the cells but induces sublethal membrane damage that alters their dielectric behavior. This change

in membrane properties provides a plausible explanation for the observed transition toward pDEP under nDEP conditions.

Finally, 5 Vpp was selected as the optimal operating condition based on a balance between cellular response and measurement reliability. In our previous work, bacterial velocity was quantified from microscopy videos by converting pixel displacement into physical distance and normalizing it to the corresponding time interval. At 3 Vpp, the limited cellular displacement reduced tracking precision and increased measurement uncertainty. In contrast, higher voltages may induce membrane perturbation, introducing additional variability and potentially altering the intrinsic electrophysiological properties of the cells. Therefore, 5 Vpp provided sufficient cellular displacement for robust image-based tracking while minimizing voltage-induced membrane perturbation. This operating condition thus offered the most reliable compromise between DEP-induced cellular movement and preservation of cell integrity

3 Principle of Dielectrophoresis

DEP refers to the movement of polarizable particles in a non-uniform electric field Buyong et al. (2018, 2014). When a bacterial cell is exposed to an alternating AC fields, charges within the cell distribute, resulting in an induced dipole. This interaction between the induced dipole and the electric field gradient generates a net force acting on the cell Pethig (2013).

The magnitude of the DEP force can be described as:

$$F_{DEP} = 2\pi\epsilon_m r^3 [CMF] \nabla E^2 \tag{1}$$

Where ϵ_m is the permittivity of the surrounding medium, r is the effective radius of the particle,*[CMF]* is the real part of the Clausius-Mossotti Factor and ∇E^2 represents the gradient of the electric field squared.

The sign *[CMF]* determines the direction of movement. A positive value results in pDEP where cells move towards regions of high electric field intensity, while a negative value results in nDEP, where cells move away from those regions.

3.1 Importance of AC frequency

The frequency of the applied AC electric field plays a critical role in determining the DEP response. This is because the CMF is frequency-dependent and reflects how the dielectric properties of the cell and surrounding medium interact at different frequencies Mortadi et al. (2016).

At low frequencies, the cell membrane dominates the polarization behavior, as it acts as an insulating layer LaLonde et al. (2015). At higher frequencies, the electric field can penetrate the membrane, and the response becomes influenced by the cytoplasm and internal structures of the cell Gagnon (2011). As a result, the DEP behavior can transition from pDEP to nDEP (or vice versa) at a specific crossover frequency.

In this study, a fixed frequency of 50 kHz was selected based on previous characterization, where both *E. coli* and *S. aureus* exhibit nDEP behavior under low-conductivity conditions Rozaini et al. (2023, 2024). This allows any deviation from

nDEP, such as the appearance of pDEP, to clearly identified as an effect of other parameters, such as applied voltage.

3.2 Importance of applied voltage

The applied voltage determines the strength of the electric field and directly influences the magnitude of the DEP force. As shown in the DEP force equation, the force is proportional to the gradient of the square of the electric field (∇E^2). Thus, increasing the voltage will result in a significantly stronger DEP force and faster bacterial movement.

In our DEP analysis, particular emphasis is placed on the movement velocity of bacterial cells. The velocity serves as a measurable parameter that reflects the DEP force acting on the cells, and therefore indirectly represents the CMF behavior Suhaimi et al. (2025). As demonstrated in our previous work, the velocity-frequency relationship retains key features of the CMF profile, allowing experimental observations to be interpreted in terms of dielectric response

To quantify this, bacterial movement was tracked from recorded microscopy videos using a video editing software. Individual cells were selected and tracked over a series of frames to obtain their positional displacement in pixel units. The displacement was then converted into real distance using the electrode gap as a reference scale, where a known physical distance (20 μm) compared to a measured pixel length in the image. The time interval was determined based on the video frame rate (25 fps), allowing the velocity to be calculated as the ratio of displacement to time Suhaimi et al. (2025). This approach provides an average velocity value for each tracked cell.

The use of velocity as a measurement parameter requires sufficient and consistent cell movement for accurate calculation. At low voltages, the DEP force is weak , resulting in slow cell movement that requires a large number of frames to obtain measurable displacement, increasing uncertainty in the calculation. In contrast, at high voltages, cells move rapidly, reducing the number of frames available for tracking and introducing potential inaccuracies due to abrupt displacement or motion blur. In addition, excessive voltage may alter the physical properties of the cells, further affecting the reliability of velocity measurements.

Therefore, the applied voltage must be carefully selected to ensure the induced DEP force produces movement without introducing any outside factors that could affect the interpretation of the results.

4 Methodology

4.1 Bacterial preparation

E. coli and *S. aureus* were used as a model for Gram-Positive and Gram-Negative bacteria in this study. Bacterial cultures were inoculated in nutrient broth (pH 7) and incubated for 5 hours. Prior to DEP experiments, the samples were diluted to a conductivity of approximately 0.03 S/m using deionized water (DI water) to ensure consistent DEP conditions. In addition, low conductivity reduces unwanted effects such as Joule heating and electrothermal flow, which can interfere with cell motion and affect experimental integrity.

4.2 DEP experiment

The DEP experiment was performed using a microelectrode device placed under an optical microscope. A small volume (1 μl) of bacterial suspension was carefully placed onto the electrode region to cover the gap, and a glass cover slip was applied to prevent evaporation.

An AC electric field was applied at a fixed frequency of 50 kHz. The voltage was varied at 3, 5, 10, 15, and 20 Vpp. Each condition was applied for 1 minute while the bacterial movement was recorded using a digital camera at 25 frames per second.

For each test, fresh bacterial sample were used as a positive controls, while heat-treated samples (80 $^{\circ}C$) were used as a negative controls to represent non-viable cells.

4.3 Analysis of DEP behavior

The DEP response was evaluated by counting the number of cells exhibiting pDEP behavior under the applied conditions. For each recorded video, a fixed number of cells was observed and categorized based on their movement toward or away from the electrode edge. The average number of pDEP cells and the corresponding standard deviation were calculated for each voltage condition.

4.4 Colony forming unit (CFU) analysis

After DEP exposure, the bacterial sample was gently flushed from the electrode using 4 μl of DI water and recollected. The collected sample was then inoculated onto nutrient agar plates and incubated at 37 $^{\circ}C$ for 24 hours.

The number of colonies formed was counted to evaluate bacterial viability. Fresh samples and heat-treated samples were included as controls in the same manner as described above.

4.5 Membrane integrity assessment (Live/Dead staining)

Membrane integrity was evaluated using a fluorescence-based Live/Dead staining method. The staining solution was prepared using calcein-AM and propidium iodide according to the manufacturer's instructions.

For fresh and heat-treated samples, 5 μl of bacterial suspension was directly mixed with the staining solution and incubated at 37 $^{\circ}C$ for approximately 2o-30 minutes in the dark. For DEP-treated samples, the recollected bacteria were mixed with the staining solution under the same conditions.

Fluorescence imaging was performed using a microscope equipped with appropriate filters. Green fluorescence indicated cells with intact membranes, while red fluorescence indicated membrane-compromised cells. Each condition was tested in five replicates to obtain a total of approximately 100 cells for analysis. The percentage of membrane-compromised against membrane-intact cells was calculated for each condition.

4.6 Transmission electron microscopy (TEM) analysis

To examine structural changes in the bacterial membrane, samples were prepared for TEM following DEP exposure. The samples were fixed and processed according to standard TEM preparation procedures.

TEM images were analyzed using ImageJ software. The membrane thickness was measured at multiple locations for each cell, and the average value was calculated for each condition. All measurement were performed using calibrated scale bars to ensure accuracy.

5 Conclusion

This study investigated the effect of applied voltage on bacterial behavior in DEP under low-conductivity conditions. An unexpected shift toward pDEP behavior was observed at higher voltages, particularly in *E. coli*, while *S. aureus* showed a much smaller response.

Further analysis confirmed that this behavior was not caused by cell death, as CFU results showed no statistically significant differences across all tested conditions. However, fluorescence staining and TEM analysis revealed a clear voltage-dependent increase in membrane damage and structural changes, indicating that the cells experienced sublethal membrane perturbation.

These findings demonstrate that high voltage can alter the dielectric properties of bacterial cells through membrane-level effects without causing immediate loss of viability. This change in membrane integrity provides a direct explanation for the observed deviation in DEP behavior. In addition, the results highlight a clear difference between *E. coli* and *S. aureus*, suggesting that bacterial structure plays an important role in determining sensitivity to electric field-induced stress.

Based on both experimental observations and analytical considerations, 5 Vpp was identified as the optimal condition for DEP analysis, providing a balance between measurable cell movement and minimal membrane disruption. Overall, this study emphasizes the importance of voltage optimization in DEP systems and provides a clearer understanding of how electric field conditions influence bacterial behavior, which is essential for achieving reliable and accurate DEP-based detection.

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Declarations

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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