Antibiotic susceptibility test based on the dielectrophoretic behavior of elongated *Escherichia coli* with cephalixin treatment

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This study reports the use of dielectrophoresis (DEP), which determined the cross-over frequency (cof) of antibiotic-induced elongation of *Escherichia coli* (*E. coli*) with regard to the rapid antibiotic susceptibility test (AST). Different dielectric properties and elongation rates of *E. coli* are caused by various concentrations of cephalixin treatment. According to the authors' results, significant changes in the cof of bacteria treated with 32 $\mu\text{g/ml}$ antibiotic for 60 min can be found by using a quadruple electrode array, and the results of DEP-based AST correspond with that of agar dilution method. Utilizing this approach could greatly reduce the period of bacteria growth, and obtain the minimum inhibition concentration of *E. coli* to cephalixin. © 2011 American Institute of Physics. [doi:10.1063/1.3600650]

The antibiotic susceptibility test (AST) is used to assess the sensitivity of pathogenic bacteria to antibiotics in clinical samples. However, drug-resistant bacteria are currently becoming more common due to the overuse of antibiotics. Conventional techniques for determining antibiotic resistance include broth dilution and disk diffusion, but the results are not available until 16–24 h or even several days later. Such delays could worsen the infection and increase the risk of hospital mortality. More rapid methods of AST are thus necessary for clinical diagnosis.

Recently, the rapid AST has recently been achieved using various developed techniques, such as microfluidics,^{1,2} electrochemistry,^{3,4} surface plasmon resonance (SPR),⁵ and dielectrophoresis (DEP).^{6,7} Chen *et al.*² demonstrated the use of the high surface-to-volume ratio microchannel made by polydimethylsiloxane for bacterial culture and rapid AST. Mann and Mikkelsen⁴ made a screen-printed carbon electrode array to detect the antibiotic susceptibility of *Escherichia coli* (*E. coli*) based on electrochemical measurement. Chiang *et al.*⁵ used the SPR biosensor to examine the drug resistance of bacteria, which were treated with the antibiotics for less than 2 h, by measuring

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the change in their optical properties. Hoettges *et al.*⁶ utilized a DEP-wall system to assess the drug resistance for bacteria and found that the significant changes in cell characteristics could be observed after 1 h. The cells or bacteria were discriminated based on their dielectric properties and morphology via the proper medium conductivity and the applied electric field. The dielectric properties and shapes of bacteria, which are drug sensitive, are changed by treating antibiotics;⁸ thus, the viability of bacteria can be distinguished.

The applications of DEP have been developing in biomedical and biotechnological sciences.^{9–11} In short, DEP is the motion of dielectric particles in a nonuniform electric field,¹² and the DEP force that acts on a spherical particle in a non-uniform electric field can be described as

$$F_{\text{DEP}} = 2\pi\epsilon_m r^3 \text{Re}[f_{\text{CM}}(\omega)] \nabla E^2, \quad (1)$$

where ϵ_m is the permittivity of the surrounding medium, E is the electric field, r is the mean radius of the particle, and the Clausius–Mossotti factor (f_{CM}), which is a function of the complex permittivity of the particle and medium, is defined as

$$f_{\text{CM}} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}. \quad (2)$$

The complex permittivity ϵ^* ($\epsilon^* = \epsilon i\sigma/\omega$) is related to the permittivity (ϵ) and conductivity (σ) via the ac field frequency (ω) for both the particle (p) and the medium (m). If particles are more polarizable than the surrounding medium, it will be attracted to the high electrical field region [positive DEP (pDEP)]; on the contrary, particles are less polarizable than the surrounding medium, and it will be repelled to the weak electrical field region [negative DEP (nDEP)]. The transition frequency point, where the behavior switches from nDEP to pDEP, is called the crossover frequency (cof).

In this study, we present a simple, rapid, and sensitive method to detect the antibiotic susceptibility of *E. coli* via measuring the changes in the DEP behavior and the elongation rate caused by the antibiotic treatment. The DEP approach was used to observe the change in dielectric properties of bacteria after the antibiotic treatment and to fix bacteria on the focal plane for scaling analysis. Various concentrations of β -lactam antibiotic cephalexin were used to treat *E. coli*, and the cell length elongated with the rising concentration. The variations in the cof and length of bacteria could be detected under the nonuniform electric field on a quadruple electrode. The results demonstrate that significant changes can be observed after 60 min after isolating bacteria from the cultural broth. The quadruple electrodes were fabricated on the glass slides (76×26 and 1 mm thick, Kimble) with a gap of 20 μm and a width of 50 μm by standard photolithography techniques and wet etching processes, as in our previous report.^{13,14}

The β -lactam antibiotics share common chemical features and include penicillin, cephalosporin, and some newer, similar agents. These antibiotics have been important in the treatment of urinary track infection (UTI), and their primary actions are inhibition of cell wall synthesis.¹⁵ The β -lactam antibiotics effect the cell elongation of *E. coli*, because penicillin-binding proteins, which are responsible for the polymerization of the peptidoglycan layer in cell wall, are inhibited or mutated.¹⁶ Most typical β -lactams inhibit cell division at low concentrations and produce cell lysis at high concentrations. *E. coli* cells would elongate by treating with various concentrations of cephalexin, and the antibiotic susceptibility could be determined based on the dielectric property of bacteria.

The shape of a bacillus is like a prolate spheroid, so the DEP force expression resembles that for a sphere.

$$F_{\text{DEP}} = \frac{2\pi}{3} ab^2 \epsilon_m^* \text{Re} \left(\frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_m^* + (\epsilon_p^* - \epsilon_m^*)L} \right) \nabla E^2, \quad (3)$$

where a and b are the radii of a prolate spheroid ($a > b$); L is the depolarization factor.^{17,18} The elongated bacilli are like needle-shaped particles, and thus the major axis of the cell is much

FIG. 1. (a) The distribution of the electric field in a quadrupole electrode was simulated by COMSOL MULTIPHYSICS and the applied voltage was $10 V_{pp}$. (b) DEP phenomena of *E. coli* cells treated with the concentrations of 0, 4, and 32 $\mu\text{g/ml}$ for 60 min under 0.6 and 4 MHz, respectively.

longer than that of the minor axis ($a \gg b$) and the depolarization factor would be approximated to $L - b^2/a^2[\ln(2a/b) - 1] \ll 1$. Therefore, the real part of the CM factor can be simplified to $\text{Re}[(\epsilon_p^* - \epsilon_m^*)/\epsilon_m^*]$. The distribution of the electric field in a quadrupole electrode is shown in Fig. 1(a). As the normal bacteria are less polarized than the medium at the proper frequency, they would be repelled to the electrode center. In contrast, they would be attracted to the electrode edge because of the length of the elongated bacteria, as shown in Fig. 1(b). In a nonuniform electric field, the induced dipole is proportional to the cell length,¹⁹ and the induced charges of the elongated cells at the ends of major axis are greater than those of the normal cells as shown in Fig. 2(a). The induced electric field of cell models with different lengths (2, 6, and 10 μm) was shown in Fig. 2(b), and the electric field of the cell at the ends is greater than that at the middle, especially the longer cell model. The background electric field would be larger, as the distance between the ends of the cell's major axis and the plane electrode became shorter due to the cell elongation. In addition, the electric field distributed in the model is like the point-plane electrode geometry.

In order to clarify the relationship between the cof and the viability, the agar dilution method was used to confirm our approach. The antibiotic susceptibility of bacteria to cephalexin was determined by the broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Cephalexin solution was added into the melted agar to prepare agar plates with various concentrations of antibiotic (1–64 $\mu\text{g/ml}$), and then *E. coli* suspension was incubated on each plate. The minimum inhibitory concentration (MIC) was determined after overnight culture. Figure 3 shows the growth of *E. coli* with various antibiotic doses, and the MIC is found to be between 32 and 64 $\mu\text{g/ml}$.

FIG. 2. (a) The distribution of the electric field of cell models with different lengths (2, 6, and 10 μm) was simulated by COMSOL MULTIPHYSICS, and the voltage was applied from end to end. (Parameters: the applied voltage of 10 V, the electrode gap of 20 μm , and the surface charge density of 0.1 C/m). (b) The magnitude of the electric field obtained in the cross section of the cell model with different length of 2 μm (solid), 6 μm (dashed) and 10 μm (dotted) as shown in the inset.

Figure 4(a) shows the variations in the cell length of the elongated *E. coli* treated with various concentrations of 1, 2, 4, 8, 16, 32, and 64 $\mu\text{g/ml}$ for 30, 60, and 120 min, respectively. *E. coli* cells were arranged straightly by DEP on indium tin oxide (ITO) quadruple electrode to quantify the length by imaging software, with the results shown in Fig. S1 in Ref. 20, and the numerical data of cell length can be seen in Fig. S2 in Ref. 20. The results show that the elongation rate increased due to the raising concentration of cephalixin. The elongation rate is clearly different when the cells were treated for over 60 min, and thus the detection time could be reduced under 120 min by discriminating the elongation rate. However, the length difference between 16 and

FIG. 3. The growth of *E. coli* using the conventional agar dilution method shows that MIC is between 32 and 64 $\mu\text{g/ml}$.

FIG. 4. The evolution of (a) elongated length and (b) cof of the elongated cells with changes in the treatment time for different antibiotic concentration ($\mu\text{g/ml}$). The means and standard errors were obtained by three repeated experiments and 10 cells were taken for analysis from each experiment.

32 $\mu\text{g/ml}$ antibiotics treatment for 60 min is not very large (~ 2 μm difference, as shown in Fig. S2 in Ref. 20). In Fig. 4(b), cofs were determined as DEP force totally turned into pDEP force. The ac electro-osmosis would be induced and the DEP force became minor at relatively lower frequency;²¹ thus, the results were not recorded below 0.2 MHz. The cof of the normal *E. coli* cells is around 3 MHz and the results show that the cof decreased as the antibiotic concentration increased. The significant change in cof can be found at 32 $\mu\text{g/ml}$ of cephalexin for 60 min treatment, and the cofs were down to 0.6 and 0.2 MHz with 60 and 120 min treatment, respectively. Thus the MIC can be determined by the change in cof after treating for 60 min, and the results are in very good agreement with those from the standard culture method (Fig. 3). However, the DEP method is much faster than the conventional method, and more sensitive than the method of elongated length measurement. According to our results, the cof of elongated bacteria thus provides a rapid and sensitive method in DEP-based AST which fits in with the results of the agar dilution method.

In summary, a rapid detection of antibiotic susceptibility was demonstrated by using the DEP chip incorporating an imaging system. The variation of the cell elongation rate can be distinguished after treating with different cephalexin concentrations over 60 min. The cof of *E. coli* cells, which were treated with 32 $\mu\text{g/ml}$ cephalexin and 60 min, showed 2.5 and 1.5 MHz differences compared to the untreated and noninhibited bacteria, respectively; thus, the cell elongation that induced the change of cof was more sensitive to the variation of cell length. In addition to the rapid detection (~ 1 h), the results of the DEP-based AST correspond to that of the standard culture method. Other Gram-negative bacilli, such as *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and so on, will be tested in our system. This rapidly diagnostic platform is a potentially useful device, not only with regard to UTI, but also other clinical infections.

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