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Development of a novel conductance-based technology for environmental bacterial sensing

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In this study, a simple impedance based technology for measuring bacterial concentrations was developed. The measurement system includes the signal amplification, copper probes and a sample loader. During the experiments, the conductance of *Bacillus subtilis* var niger, *Pseudomonas fluorescens*, and *Escherichia coli* were measured using the combination of a pre-amplifier and a lock-in amplifier. The conductance data were modeled verses the bacterial concentrations. Results indicated that the relationship between the conductance of bacterial suspensions and their concentrations follows a generic model: $Y=C_1 + C_2 \times e^{(-X/C_3)}$, where Y is the conductance (S), X is the bacterial concentration (Number/mL: abbreviated to N/mL) for all species tested, and C_{1-3} are constants. Gram negative *P. fluorescens* and *E. coli* assumed similar conductance curves, which were flatter than that of gram positive *B. subtilis* var niger. For *P. fluorescens* and *E. coli* the culturing technique resulted in higher concentration levels (statistically significant) from 2 to 4 times that measured by the impedance based technology. For *B. subtilis* var niger, both methods resulted in similar concentration levels. These differences might be due to membrane types, initial culturability and the obtained conductance curves. The impedance based technology here was shown to obtain the bacterial concentration instantly, holding broad promise in realtime monitoring biological agents.

bacterial impedance, conductance, bioaerosol, bacterial concentrations

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Bacterial detection becomes an important topic in many different fields. Traditionally, culturing technique is used to detect culturable bacterial counts, however it requires several days to obtain a result. Use of quantitative polymerase chain reaction (qPCR) can speed up the detection, however it still requires typically 4–6 h for detection with steps including sampling, DNA extraction, and amplification. In recent years, there is an increasing number of studies that aim at measuring bacterial concentrations in a real-time manner [1–3]. Among many others, the impedance-based technique has gained an increased attention. In general, the technique has been applied to detecting and/or quantifying food-borne microorganisms [1]. Recently, it has received attention from researchers in other fields due to its ability of rapid measurement for bacterial concentrations [4–7]. In some studies, the dielectrophoretic impedance measurement method was combined with antigen-antibody reaction [6,8,9]. In other studies, the electrical impedance measurements were used to detect the ionic metabolites produced during the bacterial growth [10,11]. In another study, it was applied to differentiating between dead and live cells [8].

The bacterial impedance method relies on the electrical attributes of bacterial cells and their electrophysiology [5]. In general, bacterial cells consist of adjacent structures of materials that have very different electrical properties [5].

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The cell membrane consists of a lipid bilayer rich of proteins, where the lipid molecules are organized with their polar groups and their hydrophobic hydrocarbon chains to form the membrane interior [5]. As prokaryotes, bacteria do not have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures. Accordingly, they lack a nucleus, mitochondria, chloroplasts and other organelles which are otherwise present in eukaryotic cells [12]. While the cell membrane is highly insulated, the interior of the cell is highly conductive [5]. A study has shown that the conductivity of the cell membrane is around 10^{-7} S/m, whereas that of the interior of a cell can be as high as 1 S/m [12]. The bacterial impedance based technology was shown to have detection limits of 10^2 to 10^6 cfu/mL for various bacterial cells with a detection time from 30 min to several hours [5].

Use of impedance-based technology from classic impedance microbiology to novel on-chip impedance microbiology has been thoroughly reviewed somewhere [13,14]. However, most of these studies investigated the use of the technique for detecting and/or quantifying the food-borne bacteria based on the electrophysiological and impedance properties of biological cells. Besides, the conductivity of bacterial suspensions has also been reported to study the electrical properties of bacterial cell surface and related cell surface interfacial physiology [15,16], but fewer studies have been reported to quantify the concentration of bacterial cells in the suspensions using the impedance-based method. Wheeler and Goldschmidt [17] found a positive relationship between the cell concentration of *E. coli* and the voltage changes in the suspension. They have shown a detection limit of 10^3 organisms per mL. Recently, another study has investigated the detection of bacterial cells by virtue of the impedance properties of bacterial cell suspensions using interdigitated microelectrodes [5]. The experimental results indicated that different *Salmonella* cell concentrations in the suspension resulted in different bacterial suspension impedances measured at 1 kHz. Their study suggested that the technology developed could be an alternative way for quantifying bacterial cells in the suspension in a label-free, inexpensive, and very simple manner. The detection limit was also shown comparable with many label-free immunosensors for detection of pathogenic bacteria [5]. Zhu and co-workers [18] recently reported a linear relationship between the impedance and the logarithmic value of the bacterial concentration in certain cell concentration ranges for *Porphyromonas gingivalis* and *Escherichia coli*. Different from these studies, another study has demonstrated that use of bacterial impedance together with a dielectrophoretic manipulation can differentiate between different *Bacillus* species through monitoring current change caused by different species [19]. In another study, it was demonstrated that by modifying the input frequency the impedance method can be used to quantify both viable and non-viable cells [8]. In addition, the impedance method was shown to have the capability for detection of specific bacterial species when coupled with a magnetic immunological separation method [5]. The impedance-based method has demonstrated the potential in real-time detecting and quantifying bacterial cell density in the liquid suspensions.

In this study, to further investigate the impedance-based method for quantification of bacterial concentrations in the suspension, a simpler model of the system was developed and tested on a centimeter-size chip. Three bacterial species including both Gram-negative *P. fluorescens*, *E. coli* and Gram-positive *B. subtilis* var niger were tested with the system. The relationship between the conductance and the concentrations of the bacterial suspensions were characterized and fitted mathematically using simulated equations. As an application of the technology developed, the results obtained for certain pure bacterial samples and environmental bioaerosols were compared to those obtained by the agar culturing technique.

1 Materials and methods

1.1 System setup for bacterial suspension conductance measurement

The system setup for bacterial conductance measurement is shown in Figure 1. The system is composed of three parts: signal amplification, sensing probes and a sample loader. The signal amplification includes a lock-in amplifier (LI5640, NF Corporation) and a pre-amplifier (LI-76, NF Corporation) which are connected together, the sensing probes include two identical red copper probes (the curvature radius of probe tip is 25 μm). The sample loader

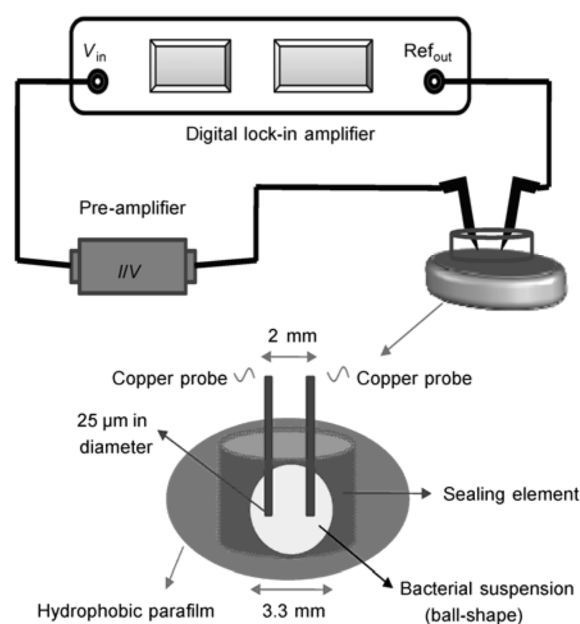

Figure 1 Experimental setup for bacterial conductance measurement technology on a centimeter-size chip; the outlet for data out is inside of the digital lock-in amplifier.

includes hydrophobic parafilm (VWR, East Fabyan Parkway, Batavia, IL), and a plastic sealing element (cylinder shape container with the top covered by parafilm). The pre-amplifier is used to amplify and convert current into voltage. The lock-in amplifier is used to amplify the signal at a fixed frequency. The parafilm is highly hydrophobic, thus water drops appear to be ball-shaped on it. The water drops can be also easily moved around the parafilm surface. The two red copper probes are connected to the reference-out of the lock-in amplifier and the pre-amplifier, respectively. The reference-out of lock-in amplifier provides the voltage between two probes. The space between the tips of the probes is fixed at about 2 mm as shown in Figure 1. The sealing element is used to minimize the liquid evaporation during the conductance measurement (8 min). The sample loader is placed onto a probe station (LPT model, Precision Systems Engineering Institute of Beijing Haida, Beijing).

1.2 Bacterial species used

In this study, *Bacillus subtilis* var niger (ATCC 9372), *Pseudomonas fluorescens* (ATCC 13525) and *Escherichia coli* (ATCC 15597) were used. *B. subtilis* var niger, a common Gram-positive bacterium, is often used as a surrogate of anthrax causing agent *Bacillus anthracis*, which is resistant to a variety of environmental stresses in the form of endospores. The endospore is formed at times of nutritional stress, allowing the organism to be resistant to environmental factors such as heat, acid, and salt and to persist in the environment for long periods of time. *P. fluorescens*, a common Gram-negative bacterium is on the other hand often used as a representative of microbial cells sensitive to stress in environmental microbial studies. A recent study showed that *P. fluorescens* caused bloodstream infections in patients diagnosed with cancer [20]. *E. coli* is another Gram-negative, rod-shaped bacterium, and its cytoplasmic membrane was shown to be substantially more enriched in cardiolipin, a dimer of phosphatidylglycerol, than previously known [21]. Here *P. fluorescens*, *B. subtilis* var niger, and *E. coli* were grown on Petri dishes with Trypticase Soy Agar (Becton, Dickson and Company, Sparks, MD, USA) under 26, 30, and 37°C, respectively for 18 h. Before the experiments, freshly purified water (Milli-Q, Millipore, Billerica, MA, USA) was added to the agar plate and colonies of studied bacterial species were removed from the agar surfaces using an inoculation loop. The resulting bacterial suspension was poured into a tube and centrifuged at 6300 rcf (Eppendorf Centrifuge 5804 R, Eppendorf, Hamburg, Germany) for 7 min. The resulting pellet was resuspended in freshly purified water and centrifuged again. The final pellet of bacteria from the second centrifugation was suspended in the freshly purified water (about 40 mL) for subsequent experiments. The final concentration levels for these three bacterial species were determined as 2×10^8, 9.28×10^9, and 2×10^9 N/mL, respectively, using a microscopic counting method.

1.3 Bacterial suspension conductance measurement

The bacterial conductance was measured using the experimental setup shown in Figure 1. During the experiment, 20 μL of bacterial suspension was pipetted onto the parafilm which was placed on the sample loader. Due to the super hydrophobic feature of the parafilm, the bacterial suspension was formed into a small ball-shaped liquid drop, which was further connected to two red copper probes spaced at ~2 mm. As observed in Figure 1, these two probes were connected to a pre-amplifier and the lock-in amplifier, respectively. The sample loader was further covered with the parafilm to minimize the liquid evaporation during the experiments. In this study, 50 mV amplitude was selected for the reference-out voltage. In another study, the same amplitude was also used [5]. For pre-amplifier, a gain factor of 10^4 was selected in this study. In order to select the optimal frequency at which the differences in conductance measurements for different bacterial species are maximized, different frequencies (1, 10, 20, 40, 60, 80, and 100 kHz) were automatically tested using a LabView computer program developed in this study. The conductance was obtained every 200 millisecond at room temperature. The bacterial conductance was compared with that of deionized water (DI) water under different frequencies. For each of the bacterial species tested, the conductance of bacterial suspension with different bacterial concentrations (five or six half-dilutions used) was measured at the frequency of 100 kHz. The conductance data continuously obtained over 8 min (total 2000 measurements) for each bacterial species were mathematically modeled. The conductance of different bacterial suspensions was measured in identical conditions, and also calibrated using quantitative polymerase chain reaction (qPCR). In this study, the electrical field generated between the two probes was also modeled using ANSYS software.

1.4 Comparison of culturing and conductance based measurement

In this study, lab-prepared bacterial suspensions including *B. subtilis* var niger, *E. coli* and *P. fluorescens* with unknown concentrations were used in comparing two quantification techniques: agar culturing and conductance based measurement. Following the method described previously, 20 μL of bacterial suspension for each species was pipetted onto the parafilm, and two probes were immersed into the liquid drop formed. The conductance of the liquid suspension for each of bacterial species was measured at the frequency identified using the system setup shown in Figure 1. The concentration of the bacterial suspension was calculated using the mathematical model developed based on the conductance for known concentrations. At the same time, culturing technique was also applied to determining the

culturable bacterial concentrations of the same bacterial suspensions. For each species, 100 μL of the bacterial liquid sample was plated onto a Trypticase Soy agar plate with three replicates and incubated at their respective cultivation temperatures as described for 18 h.

In addition, air samples of 200 L were taken using mixed cellulose ester (MCE) filters from both indoor and outdoor environments. After the sampling, the filter samples were suspended into 2 mL deionized (DI) (Millipore) water, and extracted using sonication for about 20 min in addition to a brief mixing. For culturing, 100 μL of bacterial liquid sample (extracted from filter) was plated onto agar plates with three replicates. For conductance measurement, the bacterial liquid (100 μL) mixed with 900 μL DI water went through centrifugation (6300 rcf for 5 min) twice with 900 μL supernatant taken out each time. Use of the centrifugation here allowed the removal of dissolved chemicals from the bacterial suspensions, minimizing the impact on the conductance. Following similar methods, 20 μL of bacterial liquid (after twice centrifugation) was used for conductance measurement. For each experiment, three repeats were independently conducted.

1.5 Statistical analyses

Paired *t*-test (Sigma Plot 10 component) was used to compare the results obtained by the conductance-based technique and the culturing method. The *P*-value of less than 0.05 indicated a statistically significant difference.

2 Results and discussion

A simpler bacterial conductance based technology as shown in Figure 1 was developed on a centimeter-size chip for rapidly sensing the total bacterial concentrations. Conductance curves for each of the tested bacterial species at different concentration levels were developed. Unknown concentrations for three species and environmental bioaerosols were also determined using the technology developed and the results were compared with those obtained by the culturing method. The bacterial liquid of 20 μL appeared to be a ball-shaped drop with a diameter of 3.3 mm as a result of the surface tension when pipetted onto the hydrophobic parafilm.

Figure 2 shows the modeled distribution of the electrostatic field produced between the electrodes with an applied voltage of 50 mV shown in Figure 1. In general, the electrical field was not uniform and an arc-shape field (0–234 V/m) was formed across the electrodes. As observed in Figure 2, the electrostatic field seemed stronger around the electrode than those between the electrodes. It was shown that the resolution between electrical readings was a function of the input frequency [17]. In this study, as shown in Figure 3, at the frequency of 100 kHz, the conductance differences between each of the bacteria tested and DI water was the highest. For the frequencies of larger than 10 kHz, the conductance for all bacterial species tested increased slowly compared to those measured at lower frequencies. For all species tested, the conductance was observed to increase rapidly as shown in Figure 3 when the frequency from 1 to 10 kHz was used. This was largely due to the fact that at lower frequency the double layer capacitance of the electrode dominates the total impedance. However, when the frequency is further increased above 10 kHz, the medium impedance dominates [22]. For DI water, the conductance remained similar for all frequencies tested. In a previous study, 100 kHz was also used to measure the bacterial concentrations using the impedance based technology [8].

Figure 4(a) shows the conductance data of *B. subtilis* var niger for different concentrations at the frequency of 100 kHz. In general, the conductance increased with increasing

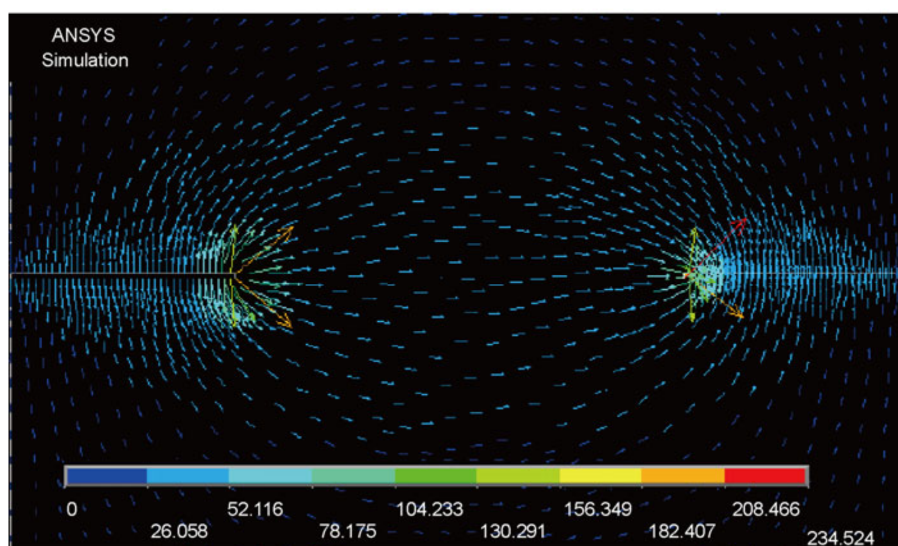

Figure 2 The electrical field (V/m) distribution generated between the copper-probes which were spaced at 2 mm with a voltage of 50 mV.

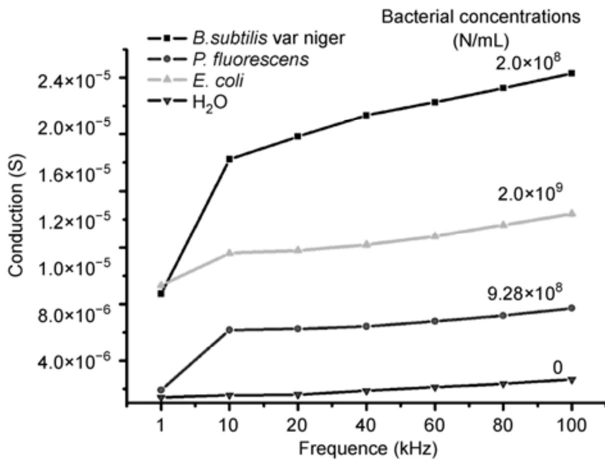

Figure 3 The conductance of bacterial suspensions and DI water measured at the frequency range of 1 to 100 kHz; the frequency was automatically switched to next one every second.

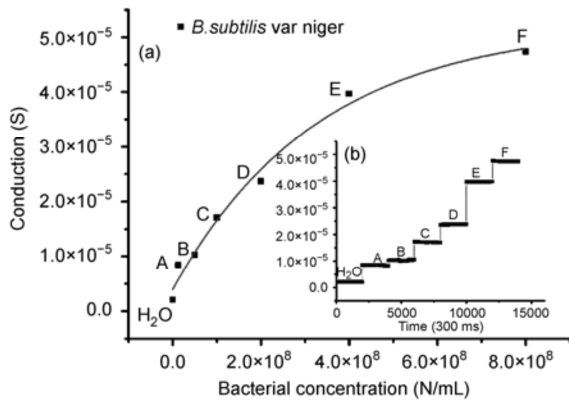

Figure 4 (a) The average conductance shown in Figure 4(b) of *B. subtilis* var niger of different concentration levels (A–F) of 0.25 ×10^8 to 8×10^8 cells/mL measured at the frequency of 100 kHz; (b) the conductance data that were obtained over an 8 min time period (total 1600 measurements); A–F refer to bacterial suspensions with different cell concentration levels investigated.

concentration as observed in Figure 4(a), but the relationship was not linear. Our qPCR tests also confirmed this when using different *B. subtilis* concentrations as observed in Figure S1. The following equation was found to best describe the relationship between the conductance and the bacterial concentrations of *B. subtilis* var niger (R^2=0.99):

$$y = 5.227\times10^{-5} - 4.830\times10^{-5}\mathrm{e}^{-(x/3.31\times10^{8})}, \quad (1)$$

where x is the bacterial concentration (N/mL) and y is the conductance of the bacterial suspension. For *P. fluorescens*, similar finding was observed as shown in Figures S2, S3 (a), but the conductance curve seemed flatter than that of *B. subtilis* var niger. The following equation was found to best fit the conductance data (R^2=0.98):

$$y = 5.49\times10^{-5} - 5.29\times10^{-5}\mathrm{e}^{-(x/5.37\times10^{9})}. \quad (2)$$

For *E. coli*, the following equation was found to best fit the data obtained (R^2=0.98)

$$y = 2.27\times10^{-5} - 1.96\times10^{-5}\mathrm{e}^{-(x/4.32\times10^{9})}. \quad (3)$$

The conductance curve for *E. coli* as shown in Figure 5(a) was similar to that of *P. fluorescens*. Similar to *B. subtilis*, our qPCR tests also confirmed the non-linear conductance-concentration relationship when measuring unknown *E. coli* concentrations as observed in Figure 6. In a previous study, it was indicated that a linear relationship was found between the impedance and the logarithmic value of the cell concentration of 10^6 to 10^{10} cfu/mL [5]. For concentration lower than 10^6 cfu/mL, the conductance values of the suspensions were shown not significantly different from the DI water [5]. In this study, for all tested bacterial species, the relationship between the suspension conductance and the cell concentration followed a similar model with different parameters regardless of the bacteria types: $Y=C_1+C_2\times \mathrm{e}^{(-X/C3)}$ where Y is the conductance (S), X is the bacterial concentration (N/mL), and $C_{1\text{-}3}$ are constants. The non-linear relationship as observed in Figures 4(a), 5(a), 6, S2(a) and S3 between the bacterial concentration and conductance level is likely attributable to two factors: (1) the non-uniform electrostatic field produced between two probes as shown in Figure 2, making the bacterial arrangement differential; (2) the differential release of ions from bacteria in response to the electrostatic field, resulting in variations in conductance levels even with the same bacterial concentration. Yang et al. [22] also suggested that the cell membrane and proteins responsible for ion transport might have played a role in the non-linear impedance change. Different from previous studies, this study has considered higher bacterial concentrations in the model developed. In this study, DI water was shown to have a conductance level of 2.05×10^{-6} S with a bacterial concentration of 0 N/mL. The data point (DI water) was also used as one data point for the simulation experiments. For those bacterial species tested in this study, the conductance levels ranged from 3×10^{-6} to 5×10^{-5} S. For

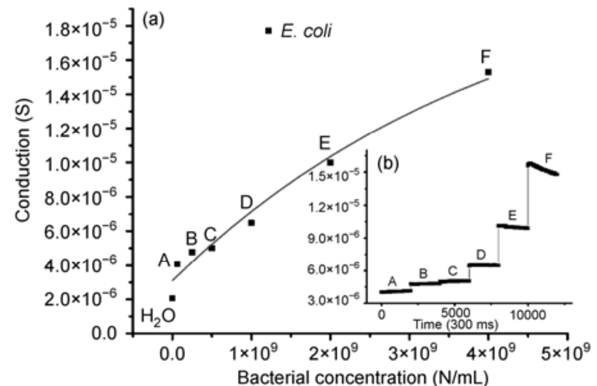

Figure 5 (a) The average conductance shown in Figure 6(b) of *E. coli* of different concentration levels (A–F) of 0.5×10^8 to 4 ×10^9 cells /mL measured at the frequency of 100 kHz; (b) the conductance data were obtained over an 8 min time period (total 1600 measurements); A–F refer to bacterial suspensions with different cell concentration levels investigated.

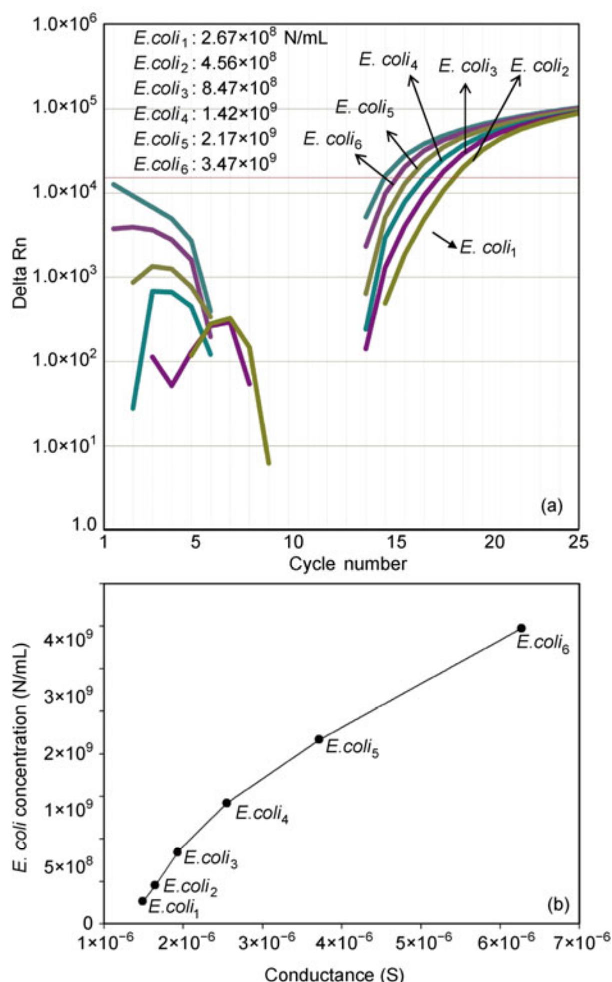

Figure 6 (a) qPCR calibration of conductance of *E. coli* with concentration levels of 2.67×10^8 to 3.47×10^9 N/mL; (b) conductance data were continuously measured over an 8 min time period for each bacterial sample (total 400 measurements).

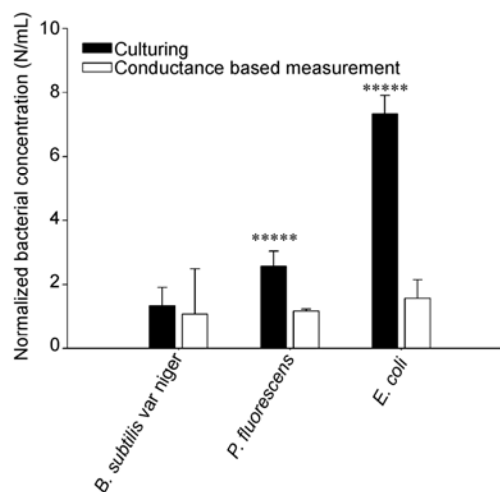

Figure 7 Comparison of liquid-borne bacterial concentrations determined using culturing (culturable counts) and conductance based techniques (total counts). Data points represent averages of three independent repeats; ***** indicates a statistically significant difference; conductance data were continuously measured over an 8 min time period for each bacterial sample(total 1600 measurements).

all tested bacterial species, the conductance levels at different concentrations remained relatively stable over 8 min as indicated in the inset figures of Figures 4(b), S2(b) and 5(b). *B. subtilis* var niger suspension was shown to have highest conductance levels followed by *P. fluorescens* and *E. coli* given similar bacterial concentrations. This might be due to the differences in surface charges for the tested bacterial species, ion releases or other unidentified factors. As discussed, the non- uniform electrical field produced between two probes might also contribute to the observed conductance trends.

Figure 7 shows comparisons of bacterial concentration levels determined using the conductance based technology developed and the culturing technique. As observed in Figure 7, for *B. subtilis* var niger the concentration levels determined by both technologies were similar (not statistically significant, *P*-value = 0.82). For *E. coli* and *P. fluorescens*, the concentration levels determined by culturing technique were higher than those determined using the conductance based technology (*P*-value = 0.002, 0.03). For *P. fluorescens*, agar culturing resulted in a concentration level about 2 times of that obtained using the conductance based technology. While for *E. coli*, about 4 times concentration levels were obtained for the culturing compared to the conductance based technology. This might be due to different bacterial membrane types, their initial culturability and the conductance relationship simulated. *E. coli* and *P. fluorescens* are Gram-negative cells and sensitive to environmental stress, while *B. subtilis* var niger is Gram-positive and stress resistant. In addition, *E. coli* and *P. fluorescens* had similar conductance curves as observed in Figures 5 and 6. The results shown in Figure 7 illustrated that conductance based technology could yield similar magnitude of estimates for *B. subtilis* concentrations, yet with an ultrafast speed. Figure 8 shows the comparisons of airborne total bacterial concentrations measured using the agar culturing and the conductance based technology. Averages of $C_{1\text{-}3}$ constants in the models developed for three pure bacterial species were used in the calculation.

As observed in Figure 8, use of the conductance based technology resulted in significantly higher bacterial concentrations up to ~10^4 times than the culturing method both in indoor and outdoor environments. Different from pure bacterial samples, the bioaerosol culturability is very low in environmental air samples. Use of conductance base technique measures both culturable and non-culturable cells, while the culturing technique measures only the culturable fraction of the total bacterial aerosols. In addition, the extraction process might have also reduced the culturable fraction of the bacteria collected on the filter. In a previous study, we have shown that the total bacterial aerosol concentration was more than 1000 times of the culturable ones through qPCR test [23]. The data obtained here by the

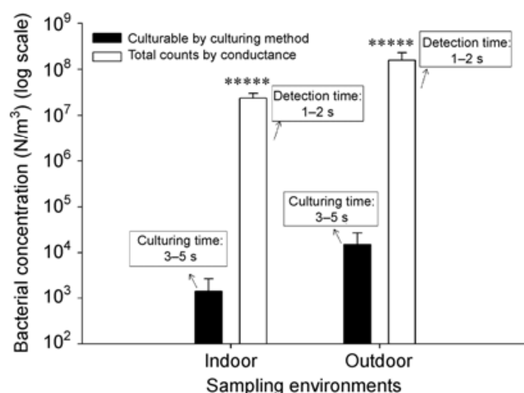

Figure 8 Comparison of airborne bacterial aerosol concentrations determined using culturing (culturable counts) and conductance based techniques (total counts) in both indoor and outdoor environments. Data points represent averages of three independent repeats and air samples were processed to remove ions using centrifugation; conductance data were continuously measured over an 8 min time period for each bacterial sample(total 1600 measurements).

conductance based technology are in line with literature data with respect to the relationship between total and culturable bacterial aerosol concentrations discussed in another study [2].

It was indicated that the conductance from the bacterial suspension was due to two contributions: ion releases from the cells and the cell surface charges [5]. When bacteria are mixed with DI water, the impedance of the suspension was observed to decrease over the time, however the impedance did not change when mixed with Phosphate Buffered Saline (PBS) buffer. It was hypothesized that PBS buffer prevented the ion release from the cells [5]. When bacterial cells are suspended into DI water, a difference in the ionic concentration between cell growth medium and DI water was produced, which could cause the cells to experience an osmotic shock. A study showed that the osmotic shock could cause the release of extracellular enzymes including invertase, a Co^{2+} activated 5′-nucleotidase, acid phosphatase, and alkaline pyrophosphatase from yeast cells [24]. The overall concentration of ions released likely depends on the bacterial concentrations in the suspension although a quantitative relationship is not known. Therefore, in this study, different bacterial concentrations could have resulted in different levels of ions. This in turn triggered different conductance levels of the bacterial suspensions. In addition, the electrical field present has introduced additional stress, e.g., membrane potential alternation, thus possibly affecting their normal metabolic activities. It is well accepted that different bacterial species vary in their amino acid, saccharide, and dipicolinic acid compositions [25]. It was suggested that the surface charges that confer hydrophilicity to spores may serve as charge carriers when an electrical potential is present, and species-specific surface charge carriers may play the role in the species-specific variations in impedance [19].

In another study, it was demonstrated that the electrical properties of *Bacillus* spores can be construed as multiple concentric shells that alternate between relatively low impedance regions (core, cortex, spore coat) and high impedance lipid regions (plasma membrane, outer membrane, exosporium) [26]. They hypothesized that AC field of frequencies below ~100 kHz are not expected to produce appreciable current across lipid membranes. Thus, in their study they attributed the conductance change to the surface charges exterior to lipid membranes. Another study pointed out that the electric charge on the surface of a bacterial cell is due to a large extent to the type of ionizable groups present on the cell surface and their spatial distribution [27]. It was also indicated that hydroxylated phospholipids phosphatidylglycerol, cardiolipin, and phosphatidylserine of the membrane components carry a net negative charge [22]. Accordingly, Yeaman and Yount [21] stated that the net charge of a bio-membrane depends largely on its phospholipid stoichiometry and architecture, and cell membranes dominated by PG, CL, or PS tend to be highly electronegative. In this study, the distance between two copper probes was about 2 mm. Beck and co-workers [19] have designed an electrical apparatus in which two electrodes were spaced about ~3 μm. In their study, they found significant differences in electrical responses when different *Bacillus* species passed through the electrodes. It was suggested that the developed system can be used to screen a variety of species according to the electrical response. In future application, a system design with movable electrodes would allow both detection at smaller electrode distance and quantification of bacterial cells in the suspension at bigger distance. However, the system might face challenges when directly measuring the bacterial concentrations in environmental samples because of the dissolved chemicals, which impacts the conductance level. Pre-treatment of the samples, e.g., filtration or centrifugation as performed in this study, thus have to be done before the analysis. The influence of chemical pollutants in the bacterial sample might be minimized when more centrifugation steps are performed. However, the contents inside bacterial cells might be also centrifuged out into the suspension when doing so. Nonetheless, the influence of the chemical pollutants can be minimized, but rather difficult to eliminate. On the other hand, filtration might be applied to removing those chemical pollutants, but the sample process afterwards requires the extraction step, which could result in damages of bacterial cells. A magnetic nanoparticle based method has been shown successful in separating cancer cells from serum samples, and it was suggested that such a method could be used in separating bacteria from complex system as well [28]. Use of antibody-coated magnetic particles could extract bacteria from the liquid suspensions, thus concentrating the species and minimizing the influence of the dissolved chemical ions on the technology developed here.

In this study, different bacterial species at similar concentration levels were shown to result in different levels of

conductance. Simulation results indicated that the nonlinear relationship between the bacterial concentration levels and the conductance can be described using a similar model as discussed here although there were some minor differences in their curve shapes. The developed technique was shown to yield similar magnitudes of estimates for some pure bacterial samples, e.g, *B. subtilis*, when compared to the traditional culturing technique. For environmental bacterial aerosols, due to their low culturable fraction, the conductance based technology obtained substantially higher total bacterial aerosol concentrations than the culturing method. It is particularly important that the developed system can monitor the bacterial concentration in a near real-time manner. When coupled with sample purification technology, the system developed here can be easily adapted to be used for monitoring bio-particle concentrations in a variety of research fields, e.g., bioaerosol, water quality and other bio-medical practices.

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1 Yang L, Bashir R. Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. Biotechnol Adv, 2008, 26: 135–150
2 Peccia J, Hernandez M. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. Atmos Environ, 2006, 40: 3941–3961
3 Peccia J, Milton D K, Reponen T, et al. A role for environmental engineering and science in preventing bioaerosol-related disease. Environ Sci Technol, 2008, 42: 4631–4637
4 Suehiro J, Ohtsubo A, Hatano T, et al. Selective detection of bacteria by a dielectrophoretic impedance measurement method using an antibody-immobilized electrode chip. Sens Actuators, B, 2006, 119: 319–326
5 Yang L. Electrical impedance spectroscopy for detection of bacterial cells in suspensions using interdigitated microelectrodes. Talanta, 2008, 74: 1621–1629
6 Yang L, Li Y, Erf G F. Interdigitated array microelectrode-based electrochemical impedance immunosensor for detection of *Escherichia coli* O157:H7. Anal Chem, 2004, 76: 1107–1113
7 Wu J, Ben Y, Chang H C. Particle detection by electrical impedance spectroscopy with asymmetric-polarization AC electroosmotic trapping. Microfluid Nanofluid, 2005, 1: 161–167
8 Suehiro J, Hamada R, Noutomi D, et al. Selective detection of viable bacteria using dielectrophoretic impedance measurement method. J Electrostat, 2003, 57: 157–168
9 DeSilva M S, Zhang Y, Hesketh P J, et al. Impedance based sensing of the specific binding reaction between Staphylococcus enterotoxin B and its antibody on an ultra-thin platinum film. Biosens Bioelectron, 10: 675–682
10 Noble P A, Dziuba M, Harrison D J, et al. Factors influencing capacitance-based monitoring of microbial growth. J Microbiol Method, 1999, 37: 51–64
11 Silleyand P, Mortimer F. In: Easter M C, ed. Rapid Microbiological Methods in the Pharmaceutical Industry. Boca Raton, Florida: CRC Press, 2003. 99
12 Pethig R, Markx G H. Applications of dielectrophoresis in biotechnology. Trends Biotechnol, 1997, 15: 426–432
13 Silley P, Forsythe S. Impedance microbiology—A rapid change for microbiologists. J Appl Bacteriol, 1996, 80: 233–243
14 Wawerla M, Stolle A, Schalch B, et al. Impedance microbiology: Applications in food hygiene. J Food Prot, 1999, 62: 1488–1496
15 Van Der Wal A, Minor M, Norde W, et al. Conductivity and dielectric dispersion of gram-positive bacterial cells. J Colloid Interface Sci, 1997, 186: 71–79
16 Wilson W W, Wade M M, Holman S C, et al. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. J Microbiol Method, 2011, 43: 153–164
17 Wheeler T G, Goldschmidt M C. Determination of bacterial cell concentrations by electrical measurements. J Clin Microbiol, 1975, 1: 25–29
18 Zhu T, Pei Z, Huang J, et al. Detection of bacterial cells by impedance spectra via fluidic electrodes in a microfluidic device. Lab Chip, 2010, 10: 1557–1560
19 Beck J D, Shang L, Li B, et al. Discrimination between Bacillus species by impedance analysis of individual dielectrophoretically positioned spores. Anal Chem, 2008, 80: 3757–3761
20 Gershman M D, Kennedy D J, Noble-Wang J. Multistate outbreak of *Pseudomonas fluorescens* bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy. Clin Infect Dis, 2008, 47: 1372–1379
21 Yeaman M R, Yount N Y. Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev, 2003, 55: 27–55
22 Yang L, Ruan C, Li Y. Detection of viable Salmonella typhimurium by impedance measurement of electrode capacitance and medium resistance. Biosens Bioelectron, 2003, 19: 495–502
23 Li K, Dong S, Wu Y, et al. Comparisons of biological contents in the air samples collected from the ground and an altitude of 238 m. Aerobiologia, 2010, 26: 233–244
24 Schwencke J, Far's G, RojasEur M. The release of extracellular enzymes from yeast by osmotic shock. J Biochem, 1971, 21: 137–143
25 Warth A D, Ohye D F, Murrel W G. The composition and structure of bacterial spores. J Cell Biol, 1963, 16: 579–592
26 Carstensen E L, Marquis R E, Child S Z, et al. Dielectric properties of native and decoated spores of *Bacillus megaterium*. J Bacteriol, 1979, 140: 917–928
27 Mainelis G, Willeke K, Baron P, et al. Electrical charges on airborne microorganisms. J Aerosol Sci, 2001, 32: 1087–1110
28 Wang D, He J, Rosenzweig N, et al. Superparamagnetic Fe_2O_3 Beads-CdSe/ZnS quantum dots core-shell nanocomposite particles for cell separation. Nano Lett, 2004, 4: 409–413

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Supporting Information

Figure S1 qPCR calibration of conductance of *B. subtilis* with concentration levels of 1.65×10^7 to 5.61×10^8 N/mL. Conductance data were continuously measured over an 8 min time period for each bacterial sample (total 1600 measurements).

Figure S2 (a) The average conductance shown in Figure 5(b) of *P. fluorescens* of different concentration levels (A–F) of 2.5×10^8 to 2.5×10^9 cells/mL measured at the frequency of 100 kHz; (b) the conductance data that were obtained over an 8 min time period; A–F refer to bacterial suspensions with different cell concentration levels investigated.

Figure S3 qPCR calibration of conductance of *P. fluorescens* with concentration levels of 1.46×10^7 to 6.96×10^8 N/mL. Conductance data were continuously measured over an 8 min time period for each bacterial sample (total 1600 measurements).

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