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Selective Detection of Live Pathogens via Surface-Confined Electric Field Perturbation on Interdigitated Silicon Transducers

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Abstract

Detection of physical changes of cells is emerging as a new diagnostic approach to determine their phenotypical features. One of such changes is related to their viability; live (viable) cells are more voluminous than the dead ones, and monitoring this parameter in tissue cells becomes essential in fields such as drug discovery and hazard evaluation. In the area of pathogen detection, an analytical system capable of specifically detecting viable cells with the simple sample preparation and detection process would be highly desirable since live microorganisms can rapidly increase their numbers even at extremely low concentration and become a severe health risk. However, current sensing strategies cannot clearly determine the viability of cells, and hence they are susceptible to false-positive signals from harmless dead pathogens. Here we developed a robust electronic immunoassay that uses a pair of polycrystalline silicon interdigitated electrodes for the rapid detection of pathogens with high specificity for live cells. After bacterial cells were specifically anchored to the surface of the antibody-modified electrode, the characteristic geometry of the transducer enables the selective detection of viable cells with a limit of detection of 3×10^2 cfu/mL and an incubation time of only 1 h. The CMOS compatible fabrication process of the chip along with the label-free, reagentless electronic detection and the easy electrode regeneration to recycle for another impedance measurement make this approach an excellent candidate for oncoming economical in-field viable-cell detection systems, fully integrable with sophisticated signal processing circuits.

In recent years, the detection of physical changes of cells as an indication of metabolic alterations caused by various stimuli such as carcinogenesis and cytotoxicity is gaining interest.^{1,2} One of the major physiological changes on cells occurs as cells become non-viable; when cells die the membrane potential breaks down, and ions in the cell interior diffuse freely to the surrounding medium, thus inducing a concomitant shrinkage of the cell.³ Therefore, an analytical system capable of finely detecting changes in the volume of cells is valuable for the determination of their viability, and therefore, for its application in industrially significant areas such as drug discovery and toxicity assessment.⁴ In addition, the application of this viability test with a simple sample preparation and detection process to the early detection of pathogenic bacterial strains would have a profound impact on other relevant fields in healthcare, food processing, water quality control, and biodefense.^{5–7} Even at extremely low concentrations live microorganisms can increase their numbers by several orders of magnitude in just a few

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SUPPORTING INFORMATION AVAILABLE

Equivalent electric circuit, FL images, viability test, variation of $-C_{sol}$ with time, SEM images of the sensor. This material is available free of charge via the Internet at <http://pubs.acs.org>.

hours, thus becoming a severe health risk. Therefore, it is desirable to develop the sensing system that can detect live pathogens on-site, sensitively, and fast enough to detect these hazardous microorganisms before they multiply for the prevention of the spread of infectious diseases. However, current sensing strategies cannot clearly determine the viability of cells, and hence they are susceptible to false positive signals from harmless dead pathogens. Recently, atomic force microscopy (AFM), electronic transducers, and optical methods have been proposed to monitor the biophysical and biomechanical changes of cells.^{8–10} These non-invasive detections via the morphological and the mechanical changes yield characteristic fingerprints for the state of cells, viable or non-viable, without using promiscuous reporters or labeling the cells with dyes. However, to apply them as practical in-field sensors, systems with faster detection, higher sensitivity, simpler analytical process, and higher selectivity are more desirable.

Here we report a robust immunoassay on impedimetric transducers that meets these requirements. As shown in Figure 1a, a pair of interdigitated electrodes was used as a transducer whose surface was functionalized by specific antibodies against a target bacterial strain. Traditionally, interdigitated electrodes have been used for studying the electric properties of thin layers and membranes because of their characteristic short electric field penetration depth.^{11,12} The short field penetration depth has also proved to be useful to monitor local changes in the electric parameters of electrolyte solutions that occur near the surface of the electrodes¹³ or to detect the presence of particular dielectric objects on the electrodes.¹⁴ In the case of the impedimetric detection of bacteria, the dielectric properties of cells can be simplified to the single-shell model; a confined electrolyte solution surrounded by a thin low-permittivity insulating shell.¹⁵ In the proper frequency range, the presence of these cells on the electrodes is expected to perturb the electric field distribution and obstruct the flow of both conduction and displacement electric currents, consequently increasing the real and imaginary parts of the impedance. Therefore, anchoring the insulating pathogens on the transducer by biomolecular recognition of antibodies should increase the resistance and decrease the capacitance of the solution between the electrodes, provided that the pathogens were voluminous enough so that the perturbation of the electric field in this region upon the cell binding became detectable (Figure 1b).

Currently, label-free bacteria biosensing with interdigitated electrodes is restricted to monitoring the presence of the pathogens as a change in the interfacial electrochemical properties of the transducer.^{16–19} Despite that this approach was successful detecting these cells, it was difficult to differentiate the presence of viable cells at the transducer interface since dead bacteria can also be recognized by antibodies and contribute to the electric signal via the change of the electrochemical properties of the interface. Moreover, the detection strategy based on interfacial changes is prone to pick up false positive signals from small proteins and lipids, which also bind on the transducer surface via non-specific binding in complex matrixes. By contrast, our approach is not susceptible to these issues since the transducer can differentiate the volume of the insulating cell via the perturbation of the surface-confined electric field measured at a proper frequency range. The interdigitated transducers have a finger width of 3 μm and pitch of 6 μm , and in this geometry 80% of electric field lines and currents are confined within a distance equal to half of the pitch.²⁰ This layout makes the detection of micrometer-sized dielectric objects such as live bacterial cells most sensitive. When the bacterial cells die, the membrane potential of cells breaks down and the ions in the cell are expelled to the outside. As a consequence, dead bacteria are less voluminous, and thus their influence on the electric field is much smaller so that they are distinguished from the live cells.²¹ Therefore, our transduction mechanism could selectively detect viable cells and be less prone to the interference from the presence of contaminants in the complex samples.

In the present work, the interdigitated electrodes used as the transducers were made of conductive polycrystalline silicon (polysilicon).²² The thin layer of native silicon oxide resulting from a spontaneous oxidation reaction can be advantageous for the sensor preparation as follows. First, the presence of this native silicon oxide layer allows one to covalently bind the recognition element to the electrode surface and the space in-between following a one-step silanization process. By this antibody functionalization, the entire interdigitated area becomes responsive to the presence of bacteria. Second, the electrodes can be easily recycled by washing them in a diluted hydrofluoric acid solution, which selectively etches the native oxide layer and thus lifts off any immobilized material without significantly damaging the polysilicon electrodes. Moreover, the mass-production of the electrodes is compatible with CMOS fabrication processes, and it is integrable with sophisticated signal processing circuits and microfluidics, making them excellent candidates for the practical laboratory-on-chip sensors.

EXPERIMENTAL METHODS

Escherichia coli cells cultured overnight were graciously donated by Prof. Yujia Xu (Hunter College-CUNY). These bacteria cells were suspended in PBS buffer after centrifuging them at 10,000 rpm for 5 min to prevent further cell growth for the concentration-dependent impedimetric detection of bacteria. The viability of the cells after this treatment was examined by culturing them again in the original growth medium (see Supporting Information). PBS buffer (0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4, tablets, Sigma) was prepared under the manufacturer's instructions. The concentration of the resulting bacteria solution was estimated by using both the MacFarland standard and the Petroff–Hausser counting chamber. To produce dead bacteria cells, an aliquot of the original live bacterial cell solution was incubated at 100 °C for 10 min. The solutions containing different cell concentrations were prepared by serially diluting the samples in PBS. Throughout this work, cfu/mL, colony forming units, is used to quantify viable bacteria, while the unit of cells/mL is applied to the dead bacteria samples and the mixed samples of live and dead bacteria.

The fabrication and the characterization of polysilicon interdigitated electrodes have been described elsewhere.²² SEM images of the sensor are available in the Supporting Information. Prior to the antibody immobilization, the electrodes were washed in a hydrofluoric acid solution (10%) for 10 s, and they were placed in a sealed gas chamber containing 3-aminopropyltrimethoxysilane overnight. After the surface of electrodes was modified with glutaraldehyde (12.5%) in a potassium carbonate solution (0.1 M, pH 8.6) for 1 h, they were rinsed with ultrapure water, and then conjugated with goat polyclonal anti-*E. coli* (100 µg/mL, Abcam) in the carbonate buffer containing sodium cyanoborohydride (50 mM). After overnight incubation, free aldehyde groups and adsorption sites on the electrodes were blocked to avoid nonspecific absorption by overnight incubation in bovine serum albumin (BSA, 1 mg/mL) and ethanolamine (50 mM) in Tris/HCl buffer (0.1 M, pH 8.6). Control sensors were prepared as above except for the addition of mouse IgG instead of the anti-*E. coli* during the immobilization process. By washing the electrodes with the hydrofluoric acid solution (10%) for 10 s, the sensors could be reused at least 10 times without noticeable loss of the performance.

Substrates for AFM imaging consisted of diced silicon wafers (1 cm²) with a 36 nm thick thermally grown silicon oxide layer modified with anti-*E. coli* as above. Live and/or dead *E. coli* cells were imaged by incubating them with the substrates in the concentration of 10⁵ cells/mL for 60 min. Subsequently, the substrates were washed with ultrapure water, dried with nitrogen, and imaged by an atomic force microscope (AFM) (3D Standalone, Asylum Research) in alternating contact mode. The radius of the silicon tips was 10 nm, and the spring constant and the resonant frequency value were 40 nN/nm and 325 kHz, respectively. The resulting AFM images were treated with WSxM software (Nanotec Electronica) to enhance the contrast.²⁷

RESULTS AND DISCUSSION

For the demonstration of the proposed transduction mechanism for the viable-specific detection of bacterial cells, the following experiments and simulations were performed. First, silicon substrates were modified with anti-*E. coli* and immersed in solutions containing either viable or dead bacterial cells (10^5 cells/mL) for 1 h. When viable bacterial cells interacted with the antibody-functionalized substrates, the characteristic rod-shape of *E. coli*, a mean length of 2 μm and a mean width of 800 nm, was bound to the antibody-coated surface as shown in Figure 2a. This dimension is consistent with previous reports.²³ When the solution containing dead bacterial cells interacted with the substrate, membrane fragments and cell debris from dead cells, whose mean height was 50 nm, were bound as shown in Figure 2b. This experiment showed that both live and dead bacteria could interact with the antibody-coated transducer, which is a common problem among existing cell sensors for the specific detection of viable cells. However, we could overcome this issue by the detection of only viable cells on impedimetric transducers as shown below.

Next, the impedimetric response for live and dead bacterial cells was tested by differential measurements with a pair of transducers. One transducer was functionalized with anti-*E. coli* (i.e., anti-*E. coli* sensor) and another transducer was modified with mouse IgG (i.e., control sensor) to compensate the background signal drift. Here the cell bindings to the sensor were confirmed by targeting them with FITC-labeled antibody (the FL images are available in the Supporting Information). With this sensor setup, impedance spectra of viable *E. coli* cells (10^5 cfu/mL) in 250 mM glycine buffer were taken in the range from 10 kHz to 1 MHz, 10 mV peak-to-peak potential at $t = 0, 20, 40, 60, 80, 100$ min with a 1260 Solartron impedance analyzer. Typically the whole impedance spectrum is obtained within 2 min with an integration time of 1 s per point. In Figure 2c, the Bode plot of the viable cell-containing solution at $t = 60$ min (red) showed an increase of the impedance modulus in the high frequency range as compared to $t = 0$ min (black). By contrast, when the same experiment was performed in a solution containing an equivalent concentration of dead bacteria, no substantial variation in the impedance between $t = 0$ min and $t = 60$ min was observed (Figure 2d). This outcome is consistent with the structural features of live and dead bacterial cells in Figures 2a and 2b because the dead bacterial cells and their fragments should interact with the electric field in much smaller degree than the live bacterial cells because of their smaller volumes.

To sensitively probe the change of the electrical properties of the transducers as the bacterial cells bind with time, the capacitance of the solution between the electrodes (C_{sol}) was obtained from the impedance spectra at each time from $t = 0$ to $t = 100$ min. To estimate the contribution of C_{sol} , the impedance spectra were fitted by the Zview2 software with the simplified equivalent circuit in Figure 2e. (Details about equivalent electric circuit are available in the Supporting Information.) It should be noted that C_{sol} for the bacterial cells was corrected by subtracting the background signal from the control sensor because the interferences from ambient factors, non-specific interactions, and parasitic components of the circuit are minimal and can be removed by this treatment (see Supporting Information). The variation of the capacitance with respect to the condition with no bacterial cells (ΔC_{sol}) was applied to probe the cell binding to the electrodes, and thus the magnitude of $-\Delta C_{\text{sol}}$ could represent the number of cells attached by the specific molecular recognition of antibody. When the value of ΔC_{sol} was plotted as a function of time, a significant difference was observed between live and dead bacteria cells as shown in Figure 2f. The values of $-\Delta C_{\text{sol}}$ of the live bacteria increased with time because more bindings of live cells to the electrodes decreased the capacitance (Figure 2f, dots). However, the values of $-\Delta C_{\text{sol}}$ for dead bacteria cells did not change with time even with the relatively high bacteria concentration and the prolonged exposure time (Figure 2f, triangles). It should be noted that $-\Delta C_{\text{sol}}$ measured by the control sensor was very small and constant even in the presence of live bacterial cells, and the binding of *E. coli* cells to the surface of the control

sensor was observed to be negligible (Supporting Information). This proves that these electrodes yield a robust background signal. Consequently, the results in Figure 2f indicate that the transduction mechanism on interdigitated electrodes is capable of detecting viable cells with high specificity. Furthermore, since the presence of insulating residues of biomaterials such as cell fragments did not significantly alter the response of the sensor, it could be applied to detect target pathogens in complex samples such as food and body fluids.

For many years, the dielectric properties of cells suspensions in aqueous solutions have been modeled with the simplified single-shell theory. In this case, the bacterial cell is considered as a conductive cytoplasm surrounded by a thin shell with low permittivity. Under an alternating electric field of frequency up to the megahertz region, the currents cannot penetrate the cell, and the impedance measurement reflects the cell volume.²⁴ When higher frequencies are applied, the cell membrane polarizes, and the dielectric property of the shell governs the measured capacitance. This phenomenon is usually referred as β -relaxation. At even higher frequencies the currents can effectively penetrate the membrane, and the impedance measurement becomes sensitive toward the heterogeneous structure and dielectric properties of the cell interior. In our system using the low frequency range, cells can be treated as insulating low-permittivity obstacles to the alternating currents, and therefore their impact on the electric field is primarily dependent on the size. Recently, the presence of insulating objects at the surface of interdigitated electrodes was reported to alter the path of the currents and electric field to increase the cell constant of the sensor with the relation,

$$k = \varepsilon / C_{\text{sol}} \quad (1)$$

where ε is the relative permittivity of the solution and C_{sol} is the capacitance associated to the solution.¹⁴ In this equation, the cell constant, k , is determined by the distribution of the electric field lines between electrodes. When insulating materials bind to the interdigitated transducer and the path of the surface-confined electric field is deviated, k is increased and thus the capacitance of the system decreases according to the eq 1. As bacterial cells are incubated in the solution containing the sensor, the variation of the capacitance not only indicates the cell binding to the electrodes, the extent of the variation also reflects the volume of bacterial cells. Since larger particles perturb the electric field more than smaller particles, it was consistent that the larger viable cells decreased the C_{sol} to a larger extent, thus resulting in the increase of $-\Delta C_{\text{sol}}$ as shown in Figure 2f. Furthermore, the time-dependent measurement of the pathogen binding on the antibody-modified substrate was possible (Figure 2f) because the increased surface coverage of bacterial cells induced the decrease of C_{sol} (i.e., the increase of $-\Delta C_{\text{sol}}$ in Figure 2f) with the same principle.

Our impedimetric measurements suggest that in a certain frequency range bacterial cells behave as rod-shaped insulators, and this behavior enables us to observe the capacitance change sensitive to the volume and the number of bacterial cells bound to the electrodes via biomolecular recognition of antibody. To support this hypothesis, we simulated the electric field line distribution between the electrodes in the presence of bacterial cells and calculated the variation of the capacitance for the sake of comparison with the experimental values. The finite-element method (FEM) was used to analyze the electric field produced by the AC potential between electrodes and the displacement currents generated in the solution. Comsol Multiphysics was used as the FEM software. The transducer was simulated as a repetition of a $3.3 \mu\text{m}$ long $\times$ $6 \mu\text{m}$ wide basic geometry including half-electrode finger at each side, yielding an electric field line distribution as shown in Figure 3a. For the simulations of the impedance variations in the presence of bacteria cells, two binding patterns of cells on the transducers were examined. These two cases were examined for the simulation because the outcomes could reveal correlation among the capacitance response and the position of bound cells. In the first

case the bacteria cells are bound to the electrode edges where the field lines are concentrated the most. In the second simulation the bacteria cells are attached between the electrodes and at the center of the electrodes where the field lines are concentrated the least. *E. coli* cells were simulated as $2\ \mu\text{m}$ long $\times$ $0.8\ \mu\text{m}$ wide octagonal prisms with null conductivity and relative permittivity of 1. The electric field distribution for the first case is shown in Figure 3b and the distribution for the second case is shown in Figure 3c. In these simulations, the dimensions of the basic geometry and the distribution and number of cells are adjusted to correspond to the total amount of cells on the transducer surface obtained for a particular experiment, and to obtain the necessary symmetries of the electric fields and currents so that insulating boundary conditions can be assigned. Under these conditions, the impedance spectra of the transducers in the presence of bacterial cells for all cases were simulated, and the significant change in the impedance spectra of the solution between the electrodes was shown as compared to the neat electrodes without cells (Figure 3d), which is in agreement with the experimental results shown in Figure 2c. This outcome supports the hypothesis that the perturbation of the surface-confined electric field by micrometer-sized insulating cells is large enough to alter the cell constant of the electrodes, and this phenomenon is the main mechanism for the specific detection of viable cells, in contrast with the traditional approach of measuring the electrochemical properties of the electrode–solution interface. By fitting the impedance spectra with the equivalent circuit (Figure 2e), the capacitance C_{sol} of panels a, b, c of Figure 3 were obtained as 151.0 pF, 136.9 pF, and 135.7 pF, respectively. These values correspond to the average capacitance decrease $-\Delta C_{\text{sol}}$ of 14.7 pF after binding of viable cells on the electrode surface, and this $-\Delta C_{\text{sol}}$ is in an excellent agreement with the experimental value, 16 pF, in Figure 2f.

Finally, the sensitivity of the proposed immunosensors was explored by measuring $-\Delta C_{\text{sol}}$ as a function of the *E. coli* concentration in solution. In this experiment, anti-*E. coli* sensors and control sensors were immersed in solutions containing viable *E. coli* cells in the concentration range from 0 to 10^5 cfu/mL for 60 min, and the ΔC_{sol} values were obtained after subtracting the impedimetric contribution of control sensors from the value of anti-*E. coli* sensors. In Figure 4, $-\Delta C_{\text{sol}}$ increased with the concentration of *E. coli* in the form of the typical sigmoid curve of an immunosensor. In this figure, error bars are obtained from the standard deviation among three different sensors, and the limit of detection was 3×10^2 cfu/mL as twice the standard deviation of the zero point. This result improves the detection limits of former impedimetric and non-impedimetric label-free approaches for the determination of pathogens,^{16,18,25} and hence it indicates that our impedimetric immunosensor is more adequate for the early determination of pathogens with high specificity for the live cells. This low detection limit is very important because the infectious dose for most pathogenic microorganisms is lower than the reported limit of detection for existing sensors,²⁶ and the improvement has been desired.

In Figure 2f, we demonstrated that the impedimetric signal for living bacterial cells was much larger as compared to the dead bacterial cells. Therefore, in theory, this sensor should be able to selectively detect live cells even under the coexistence with dead bacterial cells, as it is likely to happen in real samples. To test whether the presence of these non-viable cells could influence the sensitivity, the detection of live *E. coli* cells was examined in the presence of an excess amount of dead *E. coli* cells (10^3 cells/mL). The $-\Delta C_{\text{sol}}$ values for the live bacterial cells in this mixed sample (open circle in Figure 4) are still in excellent agreement with $-\Delta C_{\text{sol}}$ in the neat live bacteria solution (solid circle in Figure 4). This result demonstrated that the proposed impedimetric immunosensor is suitable for selectively detecting viable bacterial cells in complex samples, and hence it is not susceptible to the false-positive signals from these interferences. Therefore, this immunoassay could be easily applied for the in-field detection of live pathogens with high sensitivity.

CONCLUSIONS

In conclusion, we demonstrated that our impedimetric immunosensor could detect viable bacteria specifically with the concentration of 3×10^2 cfu/mL or higher within 1 h. The perturbation of the surface-confined electric field with the bacterial cell binding on the antibody-modified polysilicon interdigitated electrodes plays a major role for the high sensitivity and specificity of the pathogen sensor. The characteristic feature of our detection system is that by tuning the AC frequency at a certain range bacterial cells can be treated as insulating objects and their presence on the transducer can be detected as a change of the capacitance associated to the solution between the electrodes. The sensor is not susceptible to the presence of contaminants in the samples, and it could be easily adapted to the viability tests for tissue cells, which are applicable in the relevant fields of drug discovery and hazard assessment.

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Figure 1.

Illustration of the detection scheme for the impedimetric pathogen sensor: (a) functionalization of the surface of polysilicon interdigitated electrodes with the antibody provides specificity for the target pathogen; (b) the live bacterial cell binding to the antibody on the electrode perturbs the surface-confined electric field and the capacitance between the electrodes decreases, which can be detected as the positive signal for the detection. By contrast, dead bacterial cells are not voluminous enough to induce noticeable changes in the electric field lines distribution.

Figure 2.

(a) AFM image of live *E. coli* cell, scale bar = 500 nm; (b) AFM image of dead bacterial cells, scale bar = 250 nm; (c) Bode plots for anti-*E. coli* sensor before (black) and after (red) the incubation of the live bacterial cells with the concentration of 10^5 cfu/mL. (d) Bode plots for anti-*E. coli* sensor before (black) and after (red) the incubation of the dead bacterial cells with the concentration of 10^5 cells/mL. (e) The equivalent circuit used to fit impedance spectra and to obtain the capacitance values; R_c is the resistance of the contacts and the polysilicon electrodes, R_{sol} is the resistance associated to the solution between the electrodes, C_{sol} is the capacitance associated to the solution between the electrodes, and Z_{int} is the impedance of the interface; (f) variation of C_{sol} at each time from $t = 0$ to $t = 100$ min in a 10^5 cells/mL solution of live *E. coli* cells (dots) and dead *E. coli* cells (triangles). ΔC_{sol} is the variation of the capacitance with respect to the condition with no bacterial cells, which reflects the number of bacterial cells bound by antibody via biomolecular recognition.

Figure 3.

Simulations of the electric field and currents distribution around electrodes at 1 MHz (a) in the absence of pathogens, (b) in the presence of *E. coli* cells on the electrodes, (c) in the presence of *E. coli* cells at the space between the electrodes. (d) Impedance spectra obtained from these simulations; black for (a), red for (b), and blue for (c). Red lines in panels a, b, and c indicate the direction of electric field and have been arbitrarily spaced. Their density is not related to field intensity.

Figure 4.

Variation of the capacitance of the solution between the electrodes with the concentration of live *E. coli*. Error bars are based on the standard deviation obtained from three different sensors. Open dots are the variation of the capacitance with the same experiment in the presence of dead *E. coli* with the concentration of 10^3 cells/mL.