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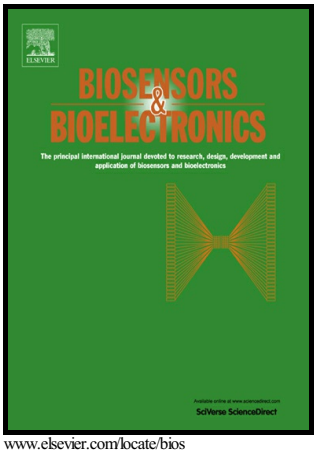

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Rapid, highly sensitive detection of Gram-negative bacteria with lipopolysaccharide based disposable aptasensor

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Abstract

Gram-negative bacteria are one of the most common microorganisms in the environment. Their differential detection and recognition from Gram-positive bacteria has been attracting much attention over the years. Using *Escherichia coli* (*E. coli*) as a model, we demonstrated on-site detection of Gram-negative bacteria by an AC electrokinetics-based capacitive sensing method using commercial microelectrodes functionalized with an aptamer specific to lipopolysaccharides. Dielectrophoresis effect was utilized to enrich viable bacteria to the microelectrodes rapidly, achieving a detection limit of 10^2 cells/mL within a 30 s' response time. The sensor showed a negligible response to *Staphylococcus aureus* (*S. aureus*), a Gram-positive species. The developed sensor showed significant advantages in sensitivity, selectivity, cost, operation simplicity, and response time. Therefore, this sensing method has shown great application potential for environmental monitoring, food safety, and real-time diagnosis.

Keywords: Gram-negative bacteria, Aptasensor, Dielectrophoresis, Capacitive biosensor, Lipopolysaccharide (LPS)

1. Introduction

Pathogenic bacterial infections and associated foodborne illnesses pose significant public health issues worldwide, and they are of special concern in developing countries, where the medical resources and public hygiene are limited. Therefore, the rapid detection and identification of these bacterial pathogens are of paramount importance for adopting treatment options and to establish adequate control measures. Gram-negative

bacteria are commonly found in the environment (Kaye et al., 2015). Their early and effective identification and differentiation from Gram-positive or other types of microorganisms have received increasing attention over the years (Neyen et al., 2016; Ichi et al., 2014).

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, and serves as a major target for the innate immune system (Ulevitch et al., 1999; Ramachandran, 2014). The recognition of LPS by macrophages (Mathison et al., 1992), granulocytes (Fang et al., 2013) and dendritic cells (Barrientos et al., 2014) and the subsequent triggering and activation of intracellular signal transduction cascade are responsible for the activation of early innate host defense against the intrusion of Gram-negative bacteria. Since LPS is a distinct pathogen-associated molecular pattern on Gram-negative bacteria, it is possible to use it as the target biomarker for Gram-negative bacteria identification.

A variety of existing LPS-based detection methods have been reviewed recently (Faraj et al., 2017). These methods include the use of LPS-specific antibodies (Levine et al., 1993; Tiirola et al., 2006), limulus-related proteins (Kawai et al., 2010), mass-spectrometry detection of lipid-A-specific fatty acids (Szponar et al., 2003) and measuring the depletion of LPS inhibitors in samples (Boelke et al, 2001). In general, these methods are often are labor-intensive and time-consuming, and require the use of sophisticated and costly instruments operated by skilled personnel. More importantly, these methods are predominately designed to measure free LPS in matrices. In clinical setting as well as for food safety inspection, unequivocally, only the number of viable bacteria reflects real pathogenic count.

To detect Gram-negative bacteria (i.e., *E. coli*), the use of LPS-specific antibodies based immunosensors has been reported (Ichi et al., 2014). However, the construction of immunosensors faces some challenges, such as the production, purification as well as the stability of antibody during and after immobilization (Trausch et al., 2017). Recently, the development and application of aptamer based biosensors have gained increasingly popularity for ultrasensitive assays (Zhu et al., 2017; Mirzajani et al., 2017; Shekari et al., 2017; Meirinho et al. 2017). The small size of aptamers, the relative molecular stability under various analytical conditions, the high affinity and specificity against target, and the capability to remain active under field-deployable conditions, make aptamer an attractive choice of capturing molecule for ultra-trace capacitive sensing (Oueslati et al., 2018; Cheng et al., 2016).

In the present work, we developed a label-free and simple-to-use biosensor to demonstrate real-time detection of viable Gram-negative bacteria (*E. coli* as representative) by AC electrokinetics-enhanced capacitive sensing (Li et al., 2014; Cui et al., 2016; Rocha et al., 2016). The sensors were modified from low cost (~$1 each), commercially-available interdigitated microelectrodes. The electrodes were functionalized with LPS-specific aptamers then blocked to achieve specificity.

Interfacial capacitance, as one of the most efficient and sensitive parameters in biosensors (Lisdat et al., 2008), was adopted to indicate the bacteria concentration. Several capacitive sensing methods have been reported for bacteria detection using different types of sensors and amplification methods. For label-free detection,

Laczka et al. reported a capacitive immunosensor using interdigitated microelectrodes, which reached a limit of detection (LOD) of 10^4-10^5 cells/mL after a response time of up to 5 hours (Laczka et al, 2008). Li et al. reported a capacitive immunosensor using an un-patterned Au electrode, which had an LOD of 220 CFU/mL and a response time of 1 h (Li, et al., 2011). To improve the detection sensitivity and reduce the response time, bacteria sensors have been integrated into flow-through systems. By placing the sensor in a continuous flow system, a capacitive sensor prepared by microcontact imprinting was reported to reach an LOD of 70 CFU/mL. However, prior to each analysis, several pre-wash steps were needed to treat the flow system. Thus, while it took only 5 minutes to measure bacteria samples, the whole analysis took over 40 minutes (Idil et al., 2017). Other biosensing methods have also been investigated for bacteria detection. Many researchers have published extensive reviews of these detection methods, including the detection of *E. coli* (Ionescu, 2017) and Gram-negative bacteria (Osei et al., 2015). Among the reviewed methods, the lowest LOD reported was 10^3 cells/mL with response times from tens of minutes to hours.

In our work, stationary sample holders were used for simple operation. To reduce the response time, this capacitive sensor uses an AC signal that can induce a microfluidic phenomenon known as dielectrophoresis (DEP) to accelerate the binding process in addition to measuring the capacitance. The DEP effect can attract bacteria to the microelectrode surface quickly. The sensor described in the present work demonstrates good sensitivity and specificity, reaching an LOD for *E. coli* of 10^2 cells/mL (~10 CFU/mL) within a 30s' response time. The sensor's response to Gram-negative bacteria (*E. coli*) can distinctly differentiate from Gram-positive bacteria (*S. aureus*) with a selectivity above 2000:1.

2. Sensing and detection mechanisms

2.1 Dielectrophoretic enrichment

Dielectrophoresis (DEP) is an efficient particle manipulation method in microfluidics. It is based on the differences in the electrical and dielectrical properties between the medium and the embedded particles (Páez-Avilés et al., 2016; Demircan et al. 2013; Pethig et al. 2010). When a particle is placed in a non-uniform electric field, the electric field strength on one side of the particle will be larger than that on the other side, leading to a net directional force on the particle, known as DEP force. Given the sizes of targets (~micron) and electrode design, DEP force should be the dominated attraction/enrichment effect against other AC electrokinetic effects for the enrichment of target analytes in this work (Castellanos et al, 2003).

The DEP velocity for a cylindrical *E. coli* particle, V_{CylDEP}, can be described as follows (Cheng et al., 2017b),

$$V_{CylDEP} = \frac{r^2 \ln\left(\frac{2l}{r}\right)}{18\eta} \varepsilon_m \,\mathrm{Re}\left\{\frac{\varepsilon_P^* - \varepsilon_m^*}{\varepsilon_m^*}\right\} \nabla |E|^2 , \quad (1)$$

where ε_m is the medium permittivity, η is the medium viscosity, r is the radius of bacteria cross section, l is the length of bacteria, $\varepsilon^* = \varepsilon - j\sigma/\omega$ is the complex permittivity with ε, σ and ω being permittivity, electrical conductivity and electric field angular frequency. Subscripts *p* and *m* denote particle and medium, respectively. $\nabla |E|^2$ is the gradient of the square of electric field strength in root mean square (RMS) value. Re[$K(\omega)$] is the real part of Clausius-Mossotian factor being limited between -0.5 and 1, and $K(\omega)$, as the Clausius-Mossi factor, is defined to be

$$K(\omega) = (\varepsilon_p^* - \varepsilon_m^*)/\varepsilon_m^* \tag{2}$$

The real part of the Clausius-Mossotti factor determines the polarity of DEP force. When positive, the particle experiences positive DEP force, moving towards high electric field regions, such as electrode edges; whereas when negative, the particles experiences negative DEP, being repelled from electrodes. In this work, positive DEP was employed for *E.coli* bacteria.

Based on Equations (1) and (2), the DEP force is dependent on AC frequency and medium's ionic strength. In this work, the background solution adopted an ionic strength similar to those used for DEP enrichment of antibodies and virus particles in prior work (Cheng et al, 2017a; Yang et al., 2017), and also close to that of environmental waters. As a result, a 100 kHz operation was used here for maximum DEP attraction.

2.2 Gram-negative bacteria (E. coli) recognition

Here, recognition of Gram-negative bacteria is based on specific binding between the aptamer and LPS. As the major outer surface membrane components present only in Gram-negative bacteria, LPS can be used as the specific target for Gram-negative bacteria detection. However, Gram-negative bacteria also secrete free LPS into the surrounding environment as a part of their physiological activities. As such, the DEP-based detection protocol needs to be designed in a way that only bacteria, and not free LPS, can be concentrated and detected in the detection time.

According to Eq. (1), V_{CylDEP} is proportional to r^2. The bacterium (*E. coli*) size is at microns scale (about 0.5 μm in radius and 2 μm in length). As LPS molecules are of nanoscale (~10 nm), free LPS will not be subject to sufficient DEP force at the voltage for cell enrichment and therefore, the consequences of free LPS binding with the aptamer probe can be neglected in this bacterial assay. Additionally, free LPS solutions were tested using this capacitive sensing method with 100 kHz AC signal from 100 to 400 mVrms. The sensor response to free LPS at different voltages and its polynomial fitting are presented in Fig. S1 in the supplementary material. The dose-response showed a quadratic dependence on voltage, which is in agreement with eq.1 for DEP dominant enrichment. The sensor response to free LPS at 5 mVrms can be estimated by extrapolation, which is negligible as expected. Since the detection time is only 30 s, binding to free LPS by passive diffusion is also negligible. To achieve specificity with respect to

other types of microorganisms, the voltage level of measuring signal needs to be chosen carefully, so that it induces negligible responses in two types of control experiments. These are, ***1)*** to test Gram-positive bacteria on functionalized sensors using *S. aureus* as a model; and ***2)*** to test *E. coli* bacteria on dummy sensors, which are blocked without LPS aptamers as described in section 3.3.

2.3 Interfacial capacitance sensing mechanisms

When electrodes are immersed in electrolytes, the resultant system can be represented as a network of surface and bulk components, both with capacitive and resistive elements (Barsoukov et al., 2005; Wu, 2008). For an AC frequency range below several hundred kHz (kiloHertz), the electric network can be further simplified as a serial connection of interfacial capacitance C_{int} and bulk resistance R_f (Cui et al., 2013; Cheng et al., 2017c), as shown in Fig.1.

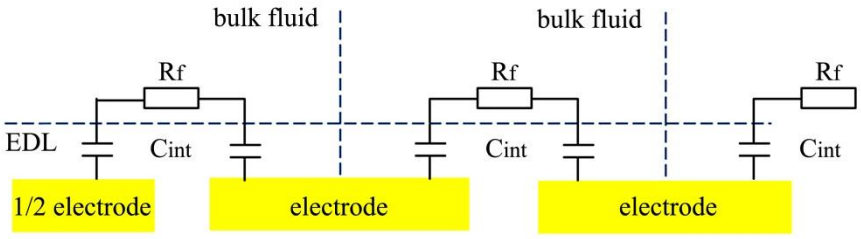


Fig. 1 The simplified equivalent circuit of electrolyte/electrode system.

An electrical double layer (EDL) appears on the surface of a conductive electrode as is exposed to a liquid electrolyte. EDL can be regarded as a parallel plate-capacitor with a dielectric layer separating two layers of charges. One layer consists of induced charges at the electrode surface, while the other layer is composed of ions attracted to the surface by the Coulomb force, electrically screening the layer of surface charges. For bare metal electrodes, the two layers of ions are separated by solvent molecules with a thickness of Debye length.

When aptamer molecules are coated on the electrode surface, the interfacial capacitance C_{int} will change since the aptamer on the electrodes increases the separation between the two layers of charges. The interfacial capacitance can be approximated as

$$C_{\text{int}} = \frac{A_{\text{int}}}{\left(\frac{D_{ap}}{\varepsilon_p} + \frac{D_{EDL}}{\varepsilon_S}\right)} \tag{3}$$

where ε_s and ε_p are the permittivities of the EDL and the aptamers. A_{int} is the electrode area, D_{ap} is the aptamer thickness and D_{EDL} represents the EDL Debye length (Lin et al., 2017).

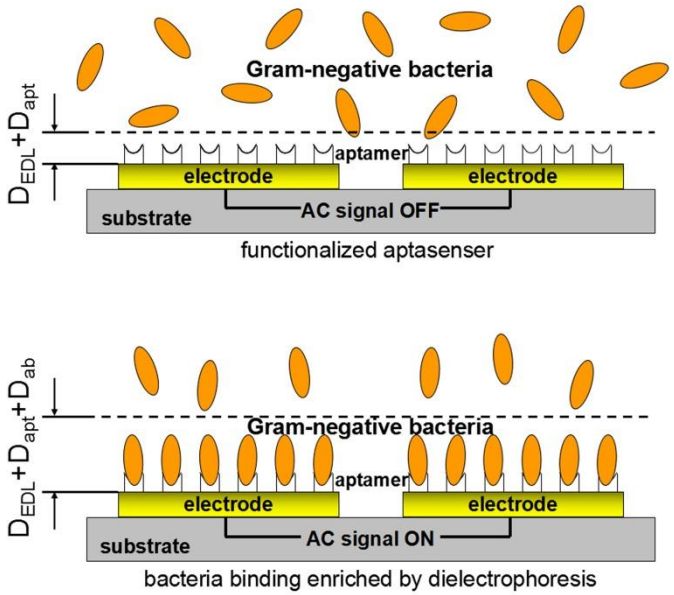


Fig. 2 Changes at the electrode surface due to the binding of specific bacteria by AC effect.

When the aptamers begin to capture bacteria, the bacteria bound on sensor surface make the surface dielectric layer thicker, as shown in Fig. 2, where the rod-shaped particles represent *E.coli* bacteria, and the concave shapes on electrodes are aptamers. The interfacial capacitance C_{int} is expected to change to

$$C_{\text{int},binding} = \frac{A_{\text{int},binding}}{\left(\frac{D_{ab}}{\varepsilon_t} + \frac{D_{ap}}{\varepsilon_p} + \frac{D_{EDL}}{\varepsilon_S}\right)} \tag{4}$$

where ε_t is the permittivity of the target particles, D_{ab} is the thickness of bacteria or other biomolecules bound with aptamers. From Eq. (4), it follows that the change in the thickness of the dielectric layer will cause a decrease in the interfacial capacitance. Assuming the electrode area remains the same before and after the binding, $A_{\text{int, }binding}$ is the effective area of the interfacial capacitor (A_{int}= $A_{\text{int, }binding}$).

In this work, the change of C_{int} was utilized to detect specific bacteria binding. Furthermore, the normalized capacitance change rate was adopted to indicate the number of bound bacteria on the electrode surface. $\Delta C/C_{int}$, as the normalized change in capacitance, is expressed as

$$\Delta C / C_{\text{int}} = (C_{\text{int},binding} - C_{\text{int}}) / C_{\text{int}} = -D_{ab} / \left(D_{ab} + \frac{\varepsilon_t}{\varepsilon_p} D_{ap} + \frac{\varepsilon_t}{\varepsilon_s} D_{DEL} \right) \tag{5}$$

By using normalized capacitance change rate, the detection errors caused by microelectrode initial impedance can be minimized. The readout is also independent of the actual working area of sensors. Consequently, good test repeatability is expected of this method.

3. Materials and procedure

3.1 Samples and reagents

The aptamer against LPS has the following sequence with 5'- thiol-modified, 5'-HS-TAGCCGGATCGCGCTGGCCAGATGATATAAAGGGTCAGCCCCCCA-3' (Thermo Fisher Scientific, Hampton, NH), selected using the LPS from *E.coli* serotype O55:B5 (Kim et al., 2012). For electrode surface blocking, 6-mercaptohexanol at 1.0 mM in deionized (DI) water was adopted to inhibit non-specific binding.

The *E. coli* bacteria DH5α strain (Thermo Fisher Scientific, Hampton, NH) was inoculated and cultured in liquid Miller's LB broth (LB) in a sterile 14 ml bacterial culture tube for 12-15 h at 37 ℃ in a 5 % CO_2: air balanced incubator. An aliquot of *S. aureus* frozen stock sample (API 6736151) kept at -80 ℃ was thawed down in a 37 ℃ water bath, streaked onto blood agar plates (BAP) in duplicate, and incubated for 16 h at 37 ℃ in a 5 % CO_2: air balanced incubator. After incubation, a single isolated colony was harvested from BAP, resuspended into 20 ml Tryptic Soy Broth (TSB), and incubated for 16 h at 37 ℃ in a 5 % CO_2: air balanced incubator. Concentrations of *E. coli* and *S. aureus* (1.256×10^9 cells/mL and 0.683×10^9 cells/mL, respectively*)* were ascertained using Petrifilm ™ aerobic plates and Petrifilm ™ reader system.

The cultured bacteria (*E. coli* or *S. aureus*) was 1:100 diluted in DI water to get Solution ***A*** with a bacteria concentration of 10^7 cells/mL, and the medium (LB or TSB) was 1: 100 diluted in DI water to get Solution ***B***. Next, solution ***A*** was diluted by solution ***B*** to get the samples with concentrations of 10^6 cells/mL, 10^5 cells/mL, 10^4 cells/mL, 10^3 cells/mL, and 10^2 cells/mL, respectively. Solution ***B*** was also used as the background for the control test. In addition, the LB medium used to culture *E. coli* to 10^9 cells/mL was centrifuged at 12,000 rpm for 5 min to remove the *E.coli* and was then 1: 100 diluted in DI water to get another control medium named LB-F, which supposedly contained free LPS molecules. All experimental culture reagents are purchased from Thermo Fisher Scientific unless otherwise stated.

3.2 Microelectrode sensors

Commercially available electrode chips, (*i.e.* surface acoustic wave resonator chips KYOCERA 433K), were modified to obtain the biosensors. The metal covers of the chips were removed as shown in Fig. S2 (a) in supplementary D:\JournalITrack\Login\ELSE\AppData\Local\youdao\dict\Application\7.5.2.0\resultui\dict\material, so that the working electrode array inside could be exposed. The scanning electron micrograph of the microelectrodes is provided in Fig. S2 (b) in supplementary D:\JournalITrack\Login\ELSE\AppData\Local\youdao\dict\Application\7.5.2.0\resultui\dict\material. The metal casing around the electrode chip forms a chamber with about 10 μL volume, serving as a holder for sample solutions. There were a pair of interdigitated electrodes made of aluminum deposited on quartz substrate. Each electrode finger was 2.0 μm wide, 170 μm long, with 1.5 μm spacing between each other. The interdigitated electrodes were electrically connected to two contact pads, which were then connected

to an impedance analyzer (Agilent 4294A).

The microelectrodes were thoroughly cleaned before probe immobilization. Following steps were performed sequentially. The chips were soaked in acetone for 20 min; rinsed with isopropyl alcohol (IPA) for 15 s; rinsed with DI water for 15 s; and dried with an air gun for 10 s. The cleaned chips were transferred into a UV Ozone cleaner and treated for 25 min to make the electrode surface more hydrophilic.

The aptamers (2 μM) were then immobilized onto the electrode surface and the chips were kept in a container under room temperature for 6 h. After immobilization, the electrodes were blocked with 1 mM 6-mercaptohexanol for 2 h at room temperature.

3.3 Detection procedure

An impedance analyzer (Agilent 4294A) was used to acquire the capacitance data. After samples (10 μL) were added into sensor chambers, the capacitance was continuously measured at 100 kHz for 30 s. To obtain an optimized voltage, different levels of AC voltages were tested first. The change rate of capacitance was calculated to indicate the adsorption level of bacteria, which is shown by slope values of normalized capacitance versus time (i.e. dC/dt [%/min]). The test was repeated three times using different chips each time. Data of dC/dt were expressed as the mean +/- standard deviations of tests run in triplicate.

Series of *E.coli* and *S. aureus* standards (1.256×10^2 to 1.256×10^6 cells/mL of *E.coli* and 0.683×10^2 to 0.683×10^6 cells/mL of *S. aureus*, respectively) were tested to evaluate the performance of the sensor. The diluted broth (solution ***B***) was used as background controls. Dummy sensors were prepared by blocking electrodes with 6-mercaptohexanol for 2 h without aptamer immobilization and used as a negative control to verify the effectiveness of the aptamer probe. Additionally, LB-F, the supernatant solution with free LPS, was also tested to evaluate any response to free LPS.

4. Results and discussion

4.1 Voltage optimization

Three voltages of 5 mVrms, 7 mVrms and 10 mVrms were tested under the same conditions of frequency and sweep time as mentioned above. An *E.coli* sample with a fixed concentration of 1.256×10^4 cells/mL was tested, and the capacitance change rates under three voltages are presented in Fig.3.

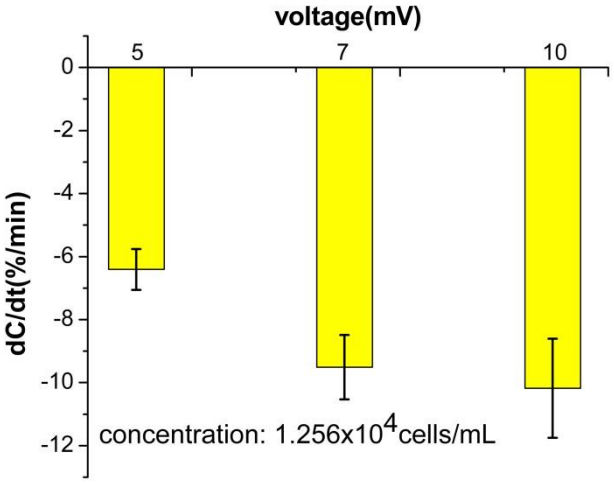


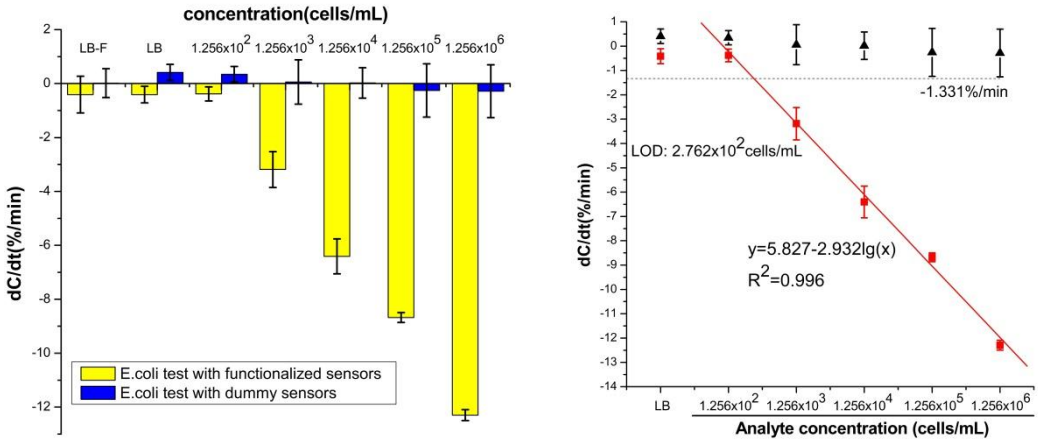
Fig. 3 dC/dt responses under different voltages for a fixed concentration of *E. coli*.

As shown in Fig. 3, a weak correlation is observed between the test voltage and dC/dt for the *E. coli* sample. The responses became more negative when the applied voltage increases from 5 mVrms to 10 mVrms. It can be observed that the increase in response from 7 mVrms to 10 mVrms was smaller than that from 5 mVrms to 7 mVrms. The examination of the transients of measured capacitance at 7 mVrms showed that the decrease in capacitance became slower with time, thus indicating a saturation of the binding sites (Cui et al., 2015). The transient curve of capacitive response is shown as Fig. S3 in the supplementary material. Once the saturation occurs, it is difficult to find a reliable correlation between dC/dt and bacteria concentration. This is also supported by the larger dC/dt deviation observed at higher voltages in Fig. 3. Based on this set of experimental data, the voltage of 5 mVrms was chosen for the subsequent experiments. In fact, unnecessarily high testing voltages will compromise the sensor specificity since strong DEP force will attract other non-targeting particles indiscriminately, including Gram-positive bacteria.

4.2 Gram-negative bacteria detection

Figure 5 (a) shows the capacitance change rates (dC/dt) in response to various concentrations of E. coli. Representative transient curves of capacitive response are provided in Fig. S4 in the supplementary material. A dose-dependent response of dC/dt was observed in the presence of *E.coli* (-0.382±0.262 %/min, -3.188 ±0.665 %/min, -6.406±0.648 %/min, -8.679±0.182 %/min, and -12.295±0.203 %/min for 1.256×10^2 cells/mL, 1.256×10^3 cells/mL, 1.256×10^4 cells/mL, 1.256×10^5 cells/mL, and 1.256×10^6 cells/mL, respectively). The LB control group showed negligible response at -0.411±0.307 %/min. More notably, the other control group of LB-F also showed negligible response of -0.406±0.681 %/min, which demonstrated that our sensor did not respond to free LPS in the medium. As for the dummy sensors, very small

responses were observed for *E.coli* concentrations from 10^2 to 10^6 cells/mL. All the values were smaller than -1.264 %/min. Therefore, it can be concluded that the responses from LPS aptasensors were produced by specific binding of *E.coli.*



(a) (b)

Fig. 4 (a) Responses of the aptasensor to the LB broth containing free LPS (LB-F), pure LB broth without LPS, and LB broth spiked with various concentrations of *E. coli*; and (b) dose response from the aptasensor as a function of *E. coli* concentration.

A linear, inverse correlation between dC/dt and *E. coli* concentration was observed in Fig. 4 (b). The dependence was expressed as $y(\%/\min) = 5.827 - 2.932 \cdot \lg x$ (bacteria concentration in cells/mL), with a Pearson correlation coefficient of R^2=0.996. The LOD was defined as 3 standard deviations from the *E. coli* background control response (LB: -0.411±0.307 %/min). The cut-off dC/dt was calculated to be -1.331%/min, which corresponded to an *E. coli* LOD of 2.762×10^2 cells/mL.

Experiments were also performed to test the applicability of this LPS aptasensor to other Gram negative bacteria. Test results showed no appreciable difference in response for three *E.coli* strains (ATCC 25922, DH5α and a field isolate). Other types of Gram negative bacteria, *Pseudomonas aerginosa* (*P. aeruginosa*), *Serratia liquefaciens* (*S. liquefaciens*), were tested along with *E. coli* and *S. aureus* isolated from mastitic cows. At a concentration of ~ 10^4 cells/mL, *P. aeruginosa* produced a slightly larger response (131%) than *E.coli*, while the response to *S. liquefaciens* was a little smaller (70%). Several factors can contribute to the magnitude of responses, such as the aptamer's ability to bind with LPS on different bacteria or bacteria size. As discussed earlier, DEP attraction force depends on particle size, and *P. aeruginosa* is slightly larger than *E. coli* and *S. liquefaciens* is slightly smaller. The *S. aureus* strain still yielded negative response. The results clearly demonstrated that the LPS sensor can effectively detect various Gram-negative bacteria, and differentiate Gram negative from Gram positive bacteria, even in different matrix. The responses to various Gram-negative bacteria against Gram-positive bacteria are given in Fig. S5 in the supplementary material. To better illustrate the performance of this sensor, Table 1 presents a comparison of performance between different electrical methods for Gram-negative bacteria detection that were reported in the literature.

Table 1 Comparison of different electrical methods for Gram-negative bacteria detection.

Device material and Detection method	Target species	Turnaround time	LOD	Label or flow system	Reference
Pt microelectrodes on glass substrates Impedance spectroscopy	*S. Typhimurium*	More than 2.5 h	10^2 CFU/mL	Yes	Pal et al., (2016)
Screen-printed interdigitated microelectrode Electrochemical	*E. coli* *S. Typhimurium*	Less than 2 h	10^2-10^3 CFU/mL	Yes	Xu et al., (2016)
Microelectrodes on silicon substrates Electrochemical impedance spectroscopy	*E.coli* *Salmonella*	Less than 5 h	10^4-10^5 cells/mL	Yes	Laczka et al., (2008)
Gold electrodes Capacitance	*E. coli* *Salmonella spp.* *S. aureus*	50 min	10^2 CFU/mL	Yes	Wannapob et al., (2010)
Quartz crystal Au electrodes Capacitance	*E. coli*	1 h	220 CFU/mL	No	Li et al., (2011)
Microcontact-*E. coli* imprinted electrodes Capacitance	*E. coli*	40 min	70 CFU/mL	Yes	Idil et al., (2017)
Aluminum microelectrodes modified from surface acoustic wave resonator chips Capacitance	*E. coli* *P. aeruginosa* *S. liquefaciens*	30 s	10^2 cells/mL (~10 CFU/mL)	No	This work

4.3 Selectivity against Gram-positive bacteria

To determine the sensor selectivity between Gram-negative and Gram-positive bacteria, we evaluated the response of sensor in the presence of *S. aureus*. As shown in Fig. 5, a response of only 1.543±0.095 %/min was observed for *S. aureus* even at a concentration of 10^6 cells/mL, which was less than half of response produced by 10^3 cells/mL of *E. coli* under the same testing conditions. The response to *S. aureus* at a concentration of 0.683×10^6 cells/mL is equivalent to that of *E. coli* at a concentration 3.261×10^2 cells/mL, demonstrating that the sensor's selectivity against Gram-positive bacteria is at a ratio of 2094:1.

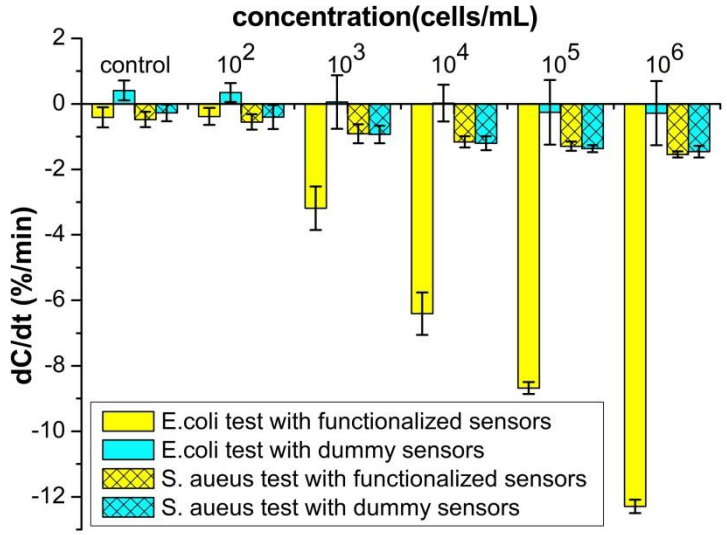


Fig. 5 Comparison of sensor responses between *E. coli* and *S. aureus.*

When testing *S. aureus*, no significant difference in response was observed between LPS aptamer functionalized sensors and dummy sensors randomization according to F test [F(1,2)=2.71, F(1,2)=1.72, F(1,2)=0.83, F(1,2)=8.37 and F(1,2)=1.21, $p<0.05$, for *S. aureus* at concentration of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively]), indicating that the LPS aptasensor responded to *E. coli* with good specificity. Since appreciable response was produced only at high concentration for *S. aureus*, the test voltage of 5 mV rms was proved again as an optimal choice.

Conclusions

In summary, we demonstrated a capacitive aptasensor based on DEP enriching effect for rapid and label-free detection of Gram-negative bacteria. A specific aptamer was adopted to capture LPS on the surface of Gram-negative bacteria. Using *E. coli* as a bacterial model, the test is completed in 30 s with an LOD of 267 cells/mL. The sensor responded similarly to three *E. coli* strains. The LPS sensor was also tested with various types of Gram-negative bacteria at ~ 10^4 cells/mL, and yielded responses within +/-30% of the standard curve of *E. coli*. This sensor has a above 2000:1 selectivity against S. aureus, a Gram-positive species, can clearly separating Gram- negative bacteria from Gram-positive bacteria. The reported sensor is a low cost modification from a commercial electrode chip. The operation is real-time and straight forward, being label-free and wash-free.

As an electrical sensor, the sensor requires the electrical conductivity of sample solution to be within a range. This work used an electrical conductivity close to that of tap water, and the obtained standard curve is also applicable to matrix with similar electrical conductivity. To apply this sensor to applications with very different fluid conductivity, the only modification is to establish a standard curve for that specific

conductivity. On the other hand, the advantages of electrical detection include miniaturization, portability, and multiplexing. This device has shown high promise to be further used in large-scale on-site applications in clinical diagnosis, environmental monitoring, and for food quality control.

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Highlights

- A lipopolysaccharide-specific aptasensor was developed for on-site detection of Gram-negative bacteria, with a sample-to-result response time of 30 seconds.
- The sensor exhibits quantitative dose response from 10^2 to 10^6 cells/mL, and reaches a limit of detection of 10^2 cells/mL of *E. coli*.
- The sensor responds similarly to three *E. coli* strains, ATCC 25922 and DH5α strain, and a field isolate.
- The sensor can differentiate *P.aeruginosa*, *S. liquefaciens* and *E. coli*, all Gram negative bacteria, from *S. aureus*, a Gram positive species.