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Research Article

Efficient and Rapid Detection of *Salmonella* Using Microfluidic Impedance Based Sensing

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We present a low cost, easy to fabricate biosensor, which can quickly and accurately detect *Salmonella typhimurium*. This study also compares the advantages of the microfluidic biosensor over a nonmicrofluidic biosensor. High density interdigitated electrode array was used to detect *Salmonella* cells inside a microfluidic chip. Monoclonal anti-*Salmonella* antibodies were allowed to be immobilized on the surface of the electrode array for selective detection of *Salmonella typhimurium*. An impedance analyzer was used to measure and record the response signal from the biosensor. The biosensor provides qualitative and quantitative results in 3 hours without any enrichment steps. The microfluidic biosensor's lower detection limit was found to be 3×10^3 CFU/mL compared to the 3×10^4 CFU/mL of the nonmicrofluidic biosensor, which shows that the microfluidic biosensor has 10-fold increased sensitivity. The impedance response of microfluidic biosensor was also significantly higher (2 to 2.9 times) compared to the nonmicrofluidic biosensor.

1. Introduction

Outbreaks caused by *Salmonella* rank at the top among all the food-borne bacterial outbreaks. The cost associated with such outbreaks is significant. Hence, rapid and accurate detection of pathogenic *Salmonella* is extremely important. *Salmonella* is estimated to cause one million illnesses each year in the United States alone, with 19,000 hospitalizations and 380 deaths [1]. The infected foods included chicken, ground beef, cucumber, and live poultry [2]. Diseases related with food-borne bacteria (excluding infections related with fungus, etc.) account for approximately 9.4 million illnesses, 55,000 hospitalizations, and 1351 deaths, which makes them a substantial threat to the safety and well-being of the population. The annual cost, directly and indirectly associated with food-borne illness, is estimated to be around $77 billion a year [3].

Salmonella typhimurium is a gram-negative bacterium that causes diarrhea, fever, and abdominal cramps and can occasionally establish localized infection in joints or even enter the human bloodstream [4]. This family of bacteria includes many serotypes, among which the outbreaks caused by *Salmonella typhimurium* and *Salmonella enteritidis* are among the most common and severe [5]. People infected with these bacteria fall victim to salmonellosis within 8 to 72 hours. Humans are infected with *Salmonella typhimurium* through consumption of contaminated food. This bacterium is mostly present in raw meat and poultry. It survives if the product is not cooked to a safe minimum internal temperature. Cross contamination is another major reason for the spread of infection when raw meat or poultry comes in contact with other foods, such as salads.

The identification and detection of food-borne pathogens continue to rely upon conventional microbiological detection techniques. Most of these conventional methods use specific agar media to separate and count bacterial cells in particular samples. These detection techniques consist of multiple steps and subprocesses which are often time consuming and take 3-4 days for initial results and up to 6-7 days for confirmation

[6]. Though these methods provide reliable data, they are not suitable for scenarios where rapid detection is the key. During the last two decades, various methods have been developed for the detection of *Salmonella typhimurium* and other food-borne pathogens. These methods include electrochemical, electrical, and optical detection using sensors. Some of these biosensors are sensitive and have rapid sensing time and low manufacturing cost and some of them can be combined with other systems to create integrated microsystems [7–17].

One popular way to detect *Salmonella typhimurium* is using impedimetric detection approach. This system was approved by the Association of Analytical Communities (AOAC) as an effective method for the detection of *Salmonella typhimurium* in food products [18, 19]. The impedance measurement is performed in between a pair of electrodes submerged in a growth medium or test solution [20]. Impedance measurements are usually carried out on interdigitated electrode arrays (IDMs) immersed in a test medium where the bacterial cells are suspended. Microelectrodes are better than conventional electrodes to carry out impedance measurements, because of low Ohmic resistance, high signal-to-noise ratio, and ability to achieve steady state quickly, and use small volumes of test sample [21]. The measured impedance consists of two primary components: the impedance due to the medium and the interface [22–25].

With the advances in micro/nanofabrication processes and in combination with biological recognition techniques, several electrochemical sensors have been fabricated for the detection of biological cells using the impedimetric technique. Impedimetric detection can be performed using label-free electrochemical impedance biosensors with nanogapped interdigitated array (IDM) microelectrodes [26, 27]. An indium-tin oxide IDM based electrochemical impedance immunosensor was used to study the bacterial growth for *Salmonella typhimurium* culture [28]. Interdigitated microelectrode based biosensors with varying electrode gaps have been developed to evaluate the effect of electrode specification on sensitivity [29]. Biosensors have been reported for impedimetric detection of *Salmonella enteritidis* in food samples using interdigitated gold electrode on silicon with detection range of 10^5 to 10^7 CFU/mL, with the detection resulting from the growth of bacteria immobilized on to the electrodes [30]. A flow cell IDM biosensor was developed to detect bacteria after enrichment in growth medium with a detection range of 8.2×10^8 CFU/mL [31]. Chemically modified single walled carbon nanotubes (SWNTs) with single stranded DNA (ssDNA) on a polished glassy carbon electrode have also been used to detect *Salmonella typhimurium* [32]. In recent years, multiple biosensors have been proposed which use DNA based sensing technologies to detect the presence of pathogen bacteria cells. In such systems, biosensing electrodes are fabricated using microstructural cystine with immobilized DNA to detect harmful pathogens like *E. coli* [33]. Another invA gene-based electrochemical DNA sensor was proposed by integrating simple DNA detection and extraction [34, 35]. Among others, label-free biosensor based on electrochemical impedance measurement in conjunction with dielectrophoretic force, biofilms on Petri dish, and gold nanoparticles on screen printed electrodes were also developed for detection and quantification of food-borne pathogenic bacteria [36–39]. The majority of the above-mentioned biosensors have low detection limit of 10^5 CFU/mL and require complex chemical process which often makes the process time consuming and expensive.

In this study we present low cost, easy to fabricate impedance microbiosensor, which can detect up to 100-fold lower concentration of bacteria cells. This biosensor is constructed using industry standard fabrication process and does not require any chemical enrichment steps, which saves time and keeps the cost down. This biosensor design provides a platform for more complex designs, which could be used to detect even lower concentration of bacteria cells in smaller time frames. We also performed comparative studies between microfluidic and nonmicrofluidic biosensor to understand the significance of the microfluidics in bacteria detection. Both platforms consist of high density interdigitated microelectrode (IDM) arrays, which were functionalized for targeted detection of *Salmonella*. The biosensor was tested with serial dilutions of *Salmonella typhimurium* cells. The study demonstrates the advantages of the microfluidic based detection platform over the nonmicrofluidic one. Both biosensors were portable and easy to fabricate with low manufacturing cost and they require no enrichment steps and have faster response time when compared to clinical methods.

2. Materials and Methods

2.1. Biosensor Design. Both biosensors consist of gold IDM array shown in Figure 1. Each sensor platform has 100 pairs of interdigitated electrodes with finger length of 0.5 mm, 15 μm width, and 10 μm spacing between them. A pair of bonding pads stretches out in opposite directions, which were used for electrical connections to the impedance analyzer. The microfluidic platform contains a fluidic channel embedded inside a polydimethylsiloxane (PDMS) slab. The channel is 2.5 mm in length, 0.5 mm wide, and 25 μm deep.

2.2. Biosensor Fabrication. The impedance biosensor was fabricated on top of a glass substrate using a series of surface micromachining, photolithography processes. The cross-sectional view of biosensor is shown in Figure 2(a). Thin layers of chromium (Cr) and gold (Au) were sputtered using magnetron RF sputtering and patterned using wet etching in potassium iodide (KI) and iodine (I_2) mixture to form the interdigitated electrode arrays.

Two polydimethylsiloxane (PDMS) covers were made and cured. The first one contains the microchannel and the second one serves as the top cover with fluidic inlet and outlet. Oxygen plasma treatment was applied on the first PDMS cover in order to change its surface to be more hydrophilic. The PDMS cover with the microchannel was then aligned and bonded to the glass substrate and baked on a hotplate at 65°C for 5 minutes by placing a heavy object on top of it. This ensures secure bonding between the PDMS and the glass substrate. The second PDMS cover with inlet

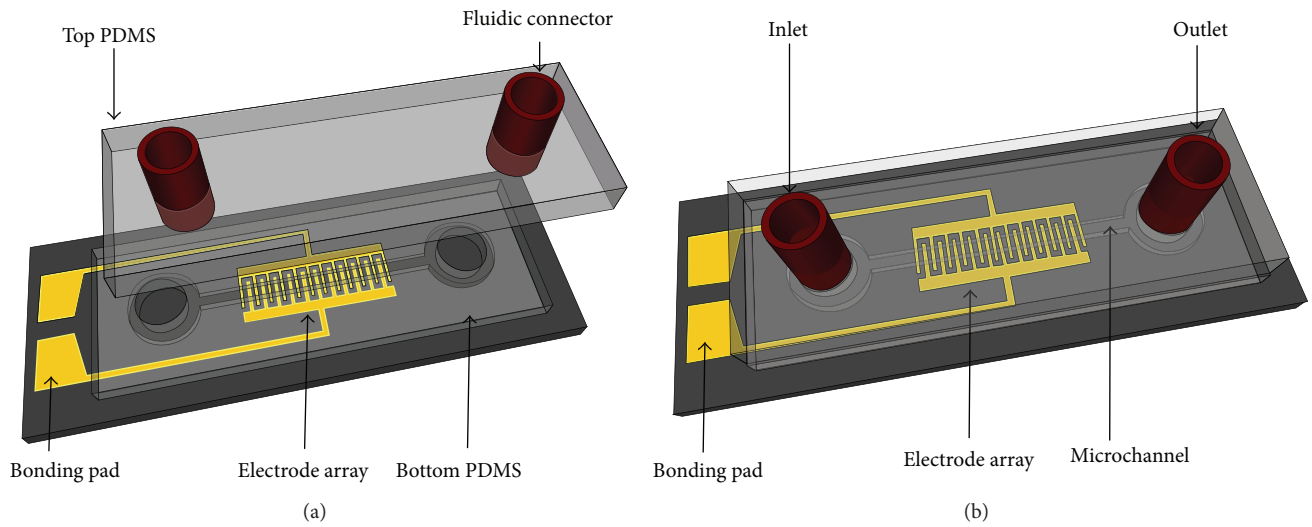


FIGURE 1: 3D schematic of the impedance biosensor showing the electrode array embedded under a microchannel with inlet and outlet.

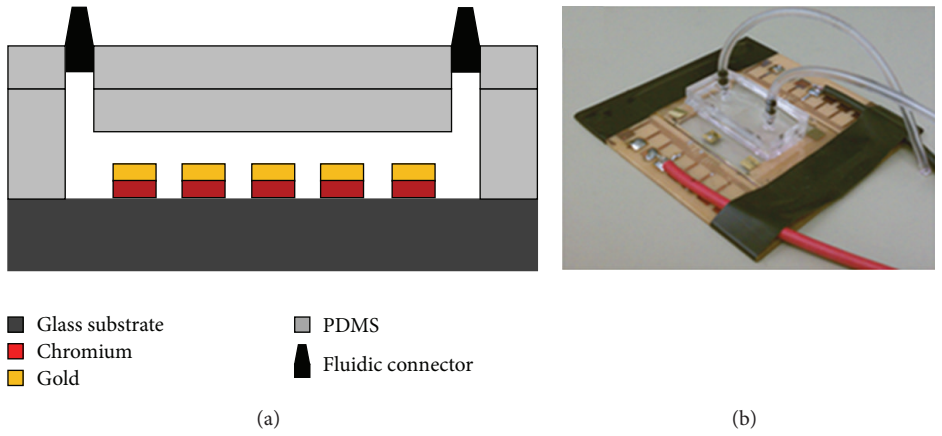


FIGURE 2: (a) Cross-sectional profile demonstrating various layers of the impedance biosensor. (b) Fabricated and packaged device.

and outlet connectors was then bonded to the first PDMS cover using oxygen plasma. The fluidic connectors were further sealed using epoxy glue in order to improve the device reliability and eliminate any possible fluid leakage. The fabricated impedance biosensor was then attached and wire-bonded to a PCB board as shown in Figure 2(b).

2.3. Culture and Cell Preparation

2.3.1. Salmonella typhimurium Broth Preparation. The preparation of the broth starts with suspending 100 g of the Bacto Peptone (Becton, Dickinson & Company, MD) into 1000 mL of distilled water. The solution was autoclaved at 121°C for 15 minutes. The *Salmonella typhimurium* (ATTC, VA) was cultured using MacConkey Agar (Remel Inc., KS). Bacteria grown on a previously cultured plate were obtained using an inoculating loop (Fisher Scientific, NH) and the broth was inoculated. The contaminated broth was incubated for about 24–36 hours before it was used.

2.3.2. Salmonella Culture Preparation. Cultured broth measuring 3 mL was centrifuged (Horizon 642VES, Drucker Company, PA) at 3200 rpm for 10 minutes. After the centrifugation, the supernatant was removed and the cells were redispersed in 3 mL PBS. The redispersed cells were centrifuged at 3200 rpm for 10 minutes and the step was repeated. The concentration of the final purified cell suspension was approximately 3×10^6 CFU/mL. This was verified by the plating method. After the centrifugation was complete, the supernatant was removed and the cells were redispersed in 200 μL PBS solution.

2.4. Antibody Immobilization. Mouse anti-*Salmonella typhimurium IgG* antibodies (Meridian Life Sciences, Inc., TN) were diluted to a concentration of 50 μgms/mL in PBS solution (Boston BioProducts, MA). This antibody concentration was determined as the optimal concentration that produced a maximum impedance change and showed the highest surface coverage, minimizing any subsequent nonspecific

adsorption. The microchannel was filled with antibody solution for 2 hours, during which the antibodies were allowed to be nonspecifically adsorbed on the surface of the gold IDM array. After 2 hours, the microchannel was cleaned with DI water to remove any unbound antibodies. Test solution containing *Salmonella typhimurium* was pumped in the microchannel with immobilized antibody. 30 minutes was allowed before the antigen was washed away. Any excess and unbound cell were further washed away using DI water, leaving the securely bonded immunoassay on the IDM array.

2.5. Data Acquisition. The impedance measurement was performed using an Agilent 4294A impedance analyzer. A sine wave of 500 mV peak voltage was applied across the terminals of the IDM arrays and the corresponding impedance values were measured for frequencies between 100 Hz and 10 MHz.

2.6. Protocol for Device Reusability. The device was reused, following a cleaning protocol that included treating the device in acetone for 30 min, followed by a wash with isopropanol and DI water, the details of which are given in our earlier publication in [16]. This cleaning protocol was used successfully for at least 20 devices over the course of the study, and the devices were each reused 5 times.

2.7. Electrical Equivalent Circuit. Equivalent circuit of the biosensors in Figure 3(a) was studied to analyze the impedance response. It consists of two double layers capacitances (C_{dl}) in series with the bulk solution resistance (R_{sol}).

The dielectric capacitance (C_{de}) of the system is assumed to be in parallel to the measurement C_{dl}-R_{sol}-C_{dl} branch [40]. When a pair electrode is immersed in an electrolyte, a very thin layer of charges is formed on the electrodes. These layers of charges align along the electrode surface and generate capacitance. This capacitance is known as double layer capacitance (C_{dl}). In the metal electrode, the test solution contributes to the resistive component (R_{sol}) in the equivalent circuit. Simulation of the equivalent circuit response was performed using EIS spectrum analyzer software. The fitting of the experimental data and simulated response (Bode plot) of equivalent circuit is demonstrated in Figure 3(b). The simulated values of C_{dl} and R_{sol} vary from 80 μF to 90 μF and from 1.58 k to 12.5 k, respectively, depending on the bacterial concentration of the test sample. Analysis of the impedance spectrum shows three distinctive regions in the impedance spectrum; these represent the response due to various components present in the equivalent circuit. Capacitive component, largely C_{dl}, dominates the spectrum at low frequencies (100 Hz–1 KHz). The 1 KHz–10 KHz region of the impedance spectrum is dependent on the response of the both resistive and capacitive components. The frequency values above 50 KHz constitute purely resistive values. The impedance of the capacitor is high at low frequencies and tends towards zero at high frequencies. The impedance response at high frequencies is largely due to the resistive component of the solution. The effect of bacterial cells is insignificant at high frequencies but at lower frequencies the impedance response which is in response to the double layer capacitance is significantly altered by the concentration of bacteria in the test solution.

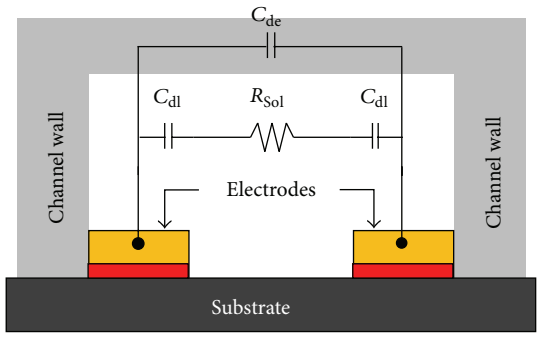
3. Results and Discussion

3.1. Dose Response and Detection. The impedance biosensor is sensitive to the variations on the surface of the electrodes. Impedance increases as antibody is immobilized on the IDM array as compared with just the control solution. Similarly, there is a change in impedance when the *Salmonella typhimurium* binds to the immobilized antibody as shown in Figure 4. For a 5–10 nm thick cell membrane, the capacitance and resistivity were found to be 0.5–1.3 μF/cm^2 and 10^2–10^5 Ω cm, respectively [41]. When bacterial cells bind to the sensor surface they do not usually come in direct contact with the surface; rather, they are actually separated by a very narrow gap (10–100 nm) [42]. The capacitive effect of the cell membrane on the interface impedance is therefore minimized, due to the aqueous gap between the membrane and the electrode surface [43, 44].

Figure 5 shows the variation in the response of the four serial concentrations of bacteria samples. The response shows that impedance increases with concentration. The lower detection limit of the biosensor was determined to be 3×10^3 CFU/mL. For all concentrations the impedance values decrease as a function of frequency.

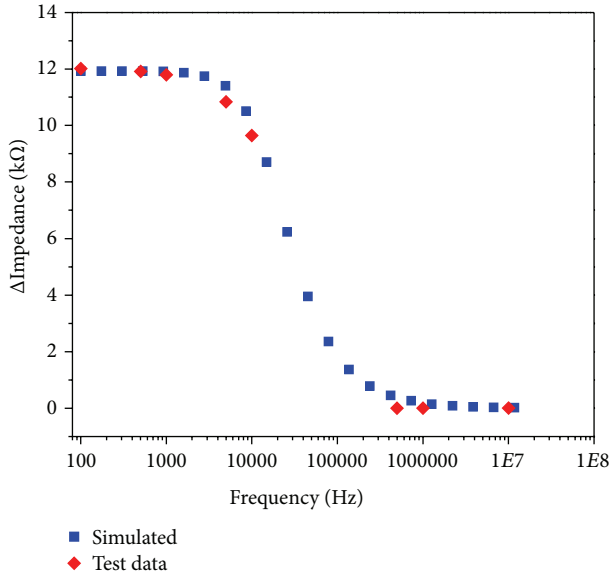
3.2. Time Response. To test the significance of time, a constant concentration (3×10^6 CFU/mL) of antigen was used. The impedance responses at different time intervals were recorded and the mean and variation in time response were plotted in Figure 6.

Impedance values at 5, 10, 30, 60, and 120 minutes were used to plot the response graph. This study was performed to investigate the minimal time required for the antigen to bind successfully with the antibody and produce a significant change in impedance response. The increase in impedance confirms the presence of *Salmonella typhimurium* in the test sample. It was observed that at 5 and 10 minutes the impedance does not increase significantly. A significant change in impedance is noted at around 30 minutes after which the impedance plateaus out. A small change in impedance is noted at 120 minutes, after which the impedance values reach equilibrium. As explained earlier, impedance response at lower frequencies largely depends on double layer capacitance and medium resistance. Considering the resistance of the medium remains the same, the increase in the double layer capacitance is a function of antibody binding. The double layer capacitance depends on several factors, including electrode potential, temperature, ionic concentrations, types of ions, and electrode surface property [45]. As the temperature and potential remained constant during this experiment, it is possible that the double layer capacitance was affected due to charged ions. Release of ions with time could account for the increased double layer capacitance and the impedance at 120 minutes. The time response study also demonstrates that the biosensor is capable of rapid detection



R_{Sol}: resistance of solution
C_{dl}: double layer capacitance
C_{de}: dielectric capacitance

(a)



(b)

FIGURE 3: (a) Equivalent circuit of the impedance biosensor demonstrating various circuit components. (b) Impedance spectrum demonstrating test data and simulated spectrum.

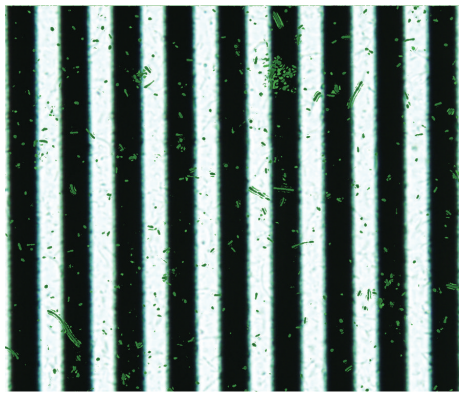


FIGURE 4: Optical image of immobilized bacteria on IDM array.

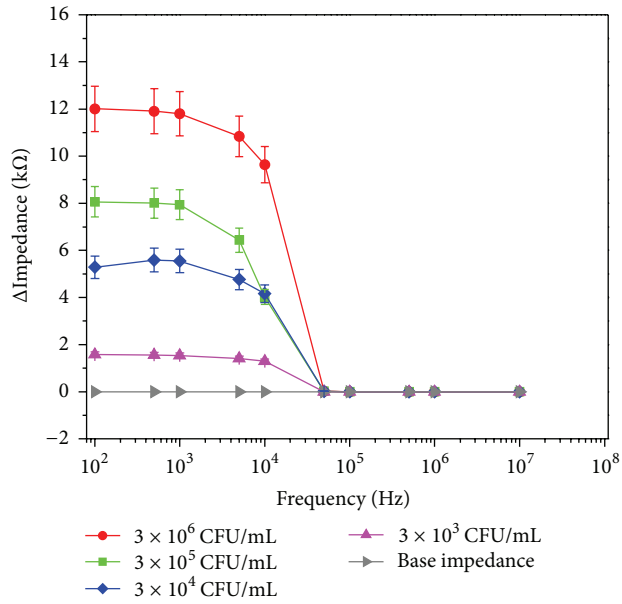


FIGURE 5: Impedance response for different concentrations of *Salmonella typhimurium*.

with an optimum binding time of ~30 minutes. It can also be inferred that extended amounts of binding time do not provide any additional advantage in the detection process. The total time required for detection is about 3 hours. This is a significant improvement over previously reported biosensors and is suitable for applications requiring rapid detection.

3.3. Negative Control Response. The biosensor was tested with a serotype of *E. coli* O157:H7 cells in order to confirm its specificity. Figure 7 indicates that impedance value obtained using the negative control was similar to the base impedance. This was expected as the sensing electrode surface was modified specifically using anti-*Salmonella typhimurium IgG* antibodies with the E. coli O157:H7 antigen. Although some *E. coli* O157:H7 cells may have nonspecifically attached to the electrode surface, their numbers were so insignificant that it did not produce a noticeable change in the impedance value. This also suggests that good antibody coverage of the electrode's surface was obtained and anti-*Salmonella typhimurium IgG* antibody does not attach to the *E. coli* O157:H7 cells. This demonstrates the specificity of the impedance biosensor in the presence of nontarget bacterial cells.

3.4. Comparison with Nonmicrofluidic Device. To understand the significance of the microfluidic impedance measurement,

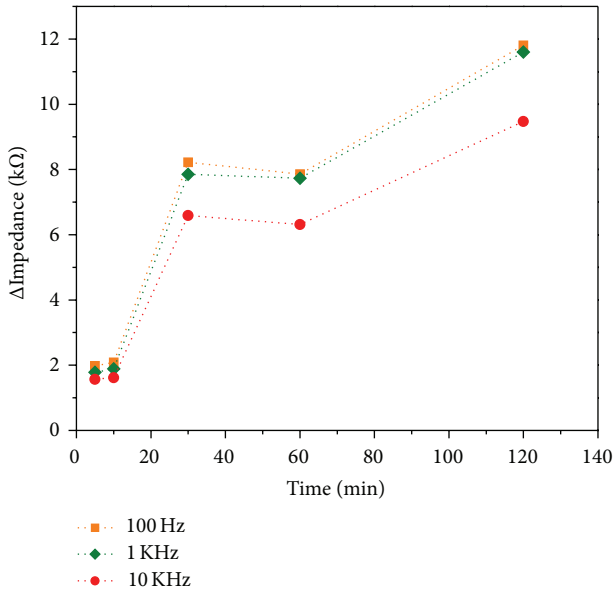


Figure 6: Variability of the sensor time response for different concentrations of *Salmonella typhimurium*.

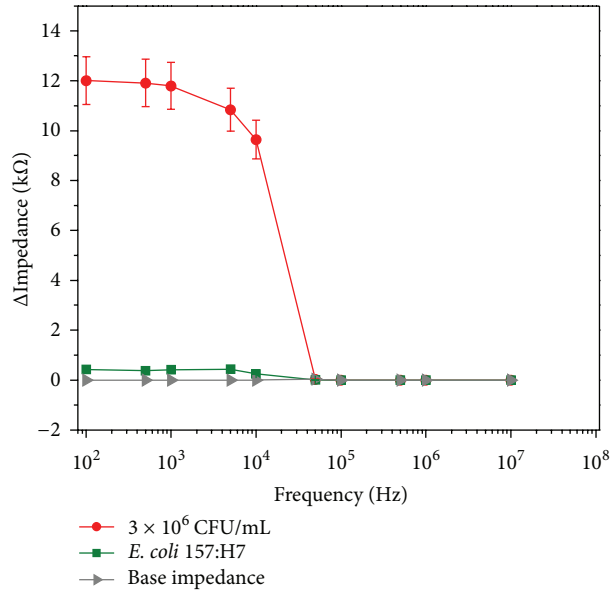


Figure 7: Comparison of *E. coli* O157:H7 impedance to *Salmonella* and base impedance.

another biosensor without the microfluidic channel was fabricated and tested. This biosensor has the same configuration as the previous microfluidic sensor. This biosensor was also tested with the three concentrations and their respective impedance responses are shown in Figure 8. It can be noted that the response of the microfluidic sensor is significantly sensitive to detection of bacteria as compared with the one without microfluidics for the same concentration in the sample. The response of the biosensor shows that the measured impedance was directly proportional to the concentration of bacteria bound to the antibody on the electrode surface. In

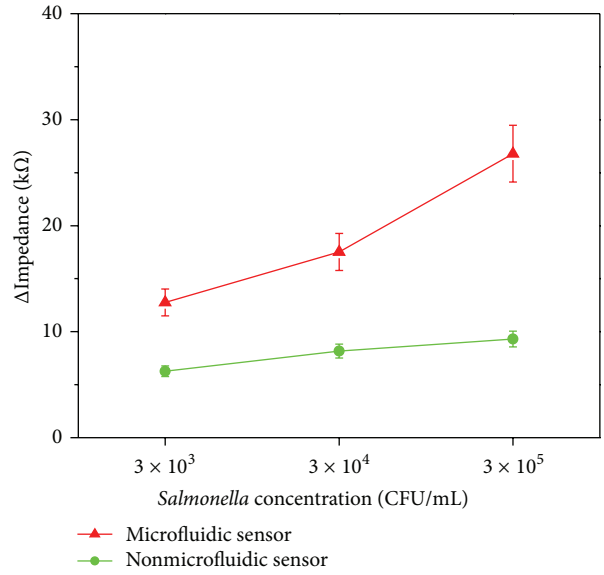


Figure 8: Comparison of impedance response at different concentrations for 1 KHz frequency.

case of the microfluidic device the bacteria sample is confined in a small volume and gets attached to the antibody on the electrode surface. This also results in higher signal-to-noise ratio. The impedance response of microfluidic impedance biosensor is significantly higher (2 to 2.9 times) than the impedance value obtained without the microfluidic channel. From the result we can infer that microfluidic channel increases the number of target cells per unit volume in the detection region, which results in an improvement in the signal level.

Also, the microfluidic impedance biosensor's lower detection limit was found to be 3×10^3 CFU/mL compared to the 3×10^4 CFU/mL of the nonmicrofluidic biosensor. The comparison shows the microfluidic impedance biosensor to have a 10-fold better sensitivity.

4. Conclusion

In this study an IDM array based impedance biosensor was successfully developed and evaluated for rapid detection of *Salmonella typhimurium*. The impedance response of microfluidic impedance biosensor was significantly higher (2 to 2.9 times) than the impedance value obtained without the microfluidic channel. From the results obtained we can infer that microfluidic channel increases the number of target cells per unit volume in the detection region, which results in improved impedance level. Also, the microfluidic biosensor's lower detection limit helps it achieve 10-fold increased sensitivity. The advantage of this sensor over other similar sensors is the specific and targeted detection of the bacteria in short amounts of time. Another advantage of this device is its reusability. This biosensor enables qualitative and quantitative detection and could potentially be used for detection of other types of bacteria by immobilizing target specific antibody.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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