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Concept for *E.coli* Detection Using Interdigitated Microelectrode Impedance Sensor

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***Abstract*— This paper presents the concept to detect *Escherichia coli* O157:H7 based on electrochemical impedance spectroscopy at interdigitated microelectrode. Interdigitated microelectrode structures was designed and fabricated, with glass as substrate material and gold electrodes. The performance of the sensors was studied by measuring the capacitance in air and impedance spectra in DI water. The feasibility of the fabricated sensor for detecting different concentrations of *Escherichia coli* in water was demonstrated. Electrochemical impedance spectroscopy (EIS) was employed as the detection technique. The impedance based response significant change for different *E.coli* concentrations in the frequency range between 1 kHz to 100 kHz.**

I. Introduction

The timely detection of pathogens is a subject of great importance. Particularly in the food industry, where goods can seldom be held during the several days required for standard methods to yield results. The use of biosensors for pathogen detection is increasingly gaining interest, and there are a number of different detection strategies and kinds of sensors [1]. Among these, the most common are the optical and electrochemical methods. In recent years steady effort has been made on the development of efficient and easy-use electrochemical biosensors. Electrochemical biosensors with rapid and highly sensitive detection capabilities of pathogens are of great demand in the field of medical and food industry. Existing immuno assay techniques such as enzyme linked immune assay (ELISA), fluoro immuno assay (FIA) and radio immuno assay (RIA) are expensive, laborious, cumbersome, hazardous and require specific labels for detection purposes thus making them more time consuming and complex [2,3,4]. Developing easy-to-use electrochemical biosensors for detecting the concentration and activities of the pathogens therefore becomes very important.

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The recent trend to miniaturize electrochemical biosensors is very attracting in the field of biosensors and has also been reported in many previous studies [5,6]. The small size of the biosensors leads to very small-scale experiments which results in very low sample volume. Hand-held electrochemical devices with accuracy and sensitivity similar to that of bench-top analyzers have already been developed for certain applications. Although still at the basic research stage, many new applications are yet to be discovered. Improved modern fabrication techniques play a major role in developing these miniaturized devices.

The use of microelectrodes in electrochemical detection has been employed in miniaturized devices [7,8]. Among microelectrodes, Interdigitated microelectrodes have drawn great attention in the area of electroanalytical chemistry in recent years by showing higher sensitivities than conventional electrodes in electrochemical measurement [9-14]. Interdigitated microelectrodes have also received great attention in the field of impedimetric immunosensing and biosensing for small molecules or DNA [15-17]. Interdigitated microelectrodes have also been used for studies of biological cell behaviors with impedance measurement [9,18]. These research results have shown the promising properties of Interdigitated microelectrodes in the area of impedance measurements and biochemical testing.

In this work we have designed and fabricated the biosensors which incorporate gold interdigitated microelectrodes, using photolithography technique. Gold has been chosen as the electrode material for this work due to its inertness. The capability of the biosensor is explained through the quantitative detection of *E.coli* in water, using the electrochemical impedance spectroscopy (EIS) as the detection technique.

II. Sensor Design and Fabrication

A. Sensor Design Configuration

Several electrode geometries have been developed [13,19] for electrochemical detection and the use of microelectrode systems exploiting their high sensitivity in conjunction with simple device set up. An interdigitated microelectrode array offers several advantages such as fast establishment of a steady-state signal, low ohmic drop of the potential, and increased signal-to-noise ratio. These advantages have led to the use of two-electrode circuits, rather than the conventional three-electrode potentiostatic circuits in wide use with macroelectrodes [20].

Interdigitated microelectrodes fabricated by lithographic technique on silicon and glass substrates have been of great interest, because they typically have a higher sensitivity than macroelectrodes. In this study, glass has been selected as the

substrate on which electrode pattern was realized. Insulating glass substrate can reduce the sensor parasitic capacitance and hence the sensitivity can be increased during measurements [21]. Research on optimization of interdigitated electrodes [19] with respect to signal-to-noise ratio has shown that the key parameters associated with the interdigitated electrode geometry are the number of electrode fingers (N), electrode finger width (w), electrode finger spacing (s) and electrode height (h). It was proved that lower number of electrodes was superior to larger number of electrodes at low concentrations of 50–100µM. Also better results were obtained with equal electrode width and spacing because of uniform electric field distribution [15, 22]. Optimum output signals were said to be obtained with electrode heights in the range of 70–140 nm. Also it has been proved that increase in height of the electrodes caused a decrease in the electric field strength and current density [23]. It has been reported by Laczka et al. [24] that smaller the electrode features, it is easier for the system to pick up on small interfacial impedance changes.

Taking all the above factors into consideration and the fact that the aim of this study is to try to lower the detection limit of the biosensor device, overall device dimensions is 3.6 cm×1.5 cm×0.07 cm with Au microelectrodes. The interdigitated microelectrode sensor would have 50 pairs of electrodes with an active sensing area of 0.4 mm^2.

B. Sensors Fabrication

The designed sensor was fabricated in-house on glass slides microfabrication and the process steps are shown briefly in Fig. 1. After cleaning the glass substrate, a standard positive photoresist film was deposited on the glass slides using a spin coater. Then, the substrate was placed on hot-plate for photoresist baking. Then the resist film was exposed to UV light through the photomask using a Karl Suss contact aligner. The UV exposed substrate was developed in developer solution. A 50 nm-thick film of gold was deposited on the photoresist-coated slides by sputtering with 2nm chromium as adhesive layer. Planar gold electrodes were then obtained on the glass substrate by removing the resist layer in acetone with ultrasound activation.

III. EXPERMENTAL

A. E.coli preparation

E. coli was grown in Luria Bertani Miller's broth overnight at 37 °C until the exponential phase was reached. The count was done spectrophotometrically and direct cell counting using Hemocytometer. The culture was then aliquoted into Eppendorf tubes and centrifuged for 10 min at 5000 rpm. After the centrifugation was complete, the supernatant was discarded and the *E.coli* sediment was re-dispersed in sterilized DI water; then this was serially (1:10) diluted with DI water to desirable concentrations for impedance measurements.

B. Electrical impedance spectroscopy (EIS) study

The device for electrical impedance measurements consists of interdigitated microelectrode and a chamber (~1ml capacity) right above the electrode formed by polystyrene. IM-6 impedance analyzer (Zahner-elektrik CMBH & Co. KG, Germany) was used to measure the impedance response of the biosensor from 1Hz- 1MHz frequency. A 100mV sinusoidal signal is used as the excitation signal to measure the impedance. For measurements, 900µl of sample was placed into the chamber and covered with a glass cover. One of the two microband array electrodes was connected to the test and sense probes, and the other was connected to the reference and counter electrodes on the IM-6 impedance analyzer.

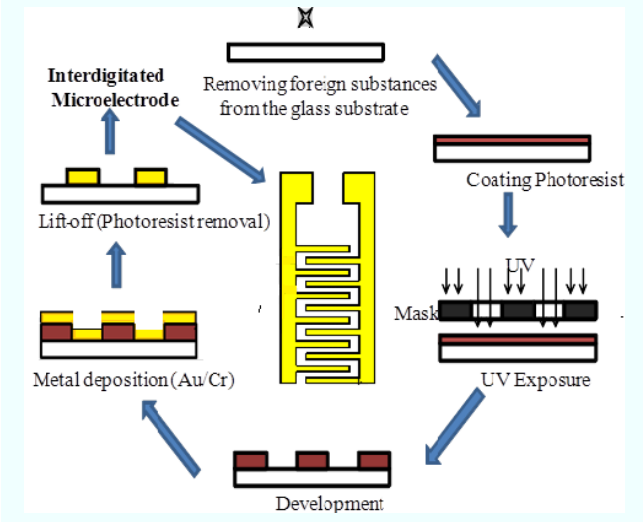


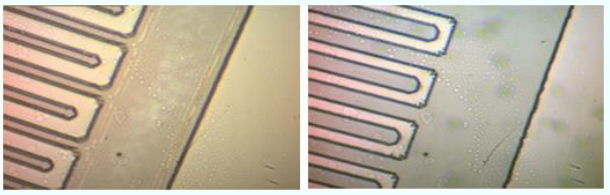
Figure 1. Microfabrication process steps.

IV. RESULTS AND DISCUSSION

A. Development artifact during sensor fabrication

To produce the photoresist pattern on substrate, UV-exposure time was fixed to get equal electrode width and spacing according to our design. If the exposure time is low or high, desired pattern dimensions cannot be achieved called exposure artifact and is shown in Fig. 2a, Fig. 2b. With low UV exposure time the electrode width and spacing were 2 µm, 16 µm respectively. With long exposure time wider electrode width of 15 µm was achieved.

The exposed photoresist portion was removed by immersion in a developer solution for a fixed period of time. Under-development caused the cloudy parts on the photoresist developed pattern and after lift-off the metal deposited on this cloudy part resulted in metal (gold) as shown in Fig. 3a. Over-development caused the loss of some parts on the photoresist developed pattern as shown in Fig. 3b.



(a) (b)

Figure 2. Optical microscopic images of exposure artifact (a) low UV-exposure time, (b) high UV-exposure time

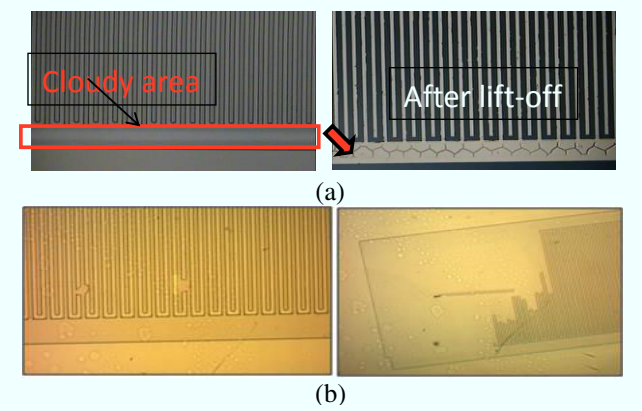


Figure 3. Optical microscopic images of photoresist development artifact (a) under-development, (b) over-development

B. *Capacitance characterization of sensors in air*

The stability of the sensor under AC electric field was studied by measuring the capacitance in air at room temperature. Capacitance value of the interdigitated microelectrode sensors was calculated theoretically by using the formula (1) proposed by Van Gerwan et al. [15], as in

$$C = nl\varepsilon_0\varepsilon_r/2 \quad (1)$$

where 'C' is the generated capacitance (F), 'n' is number of finger electrodes, '*l*' is length of finger electrodes and 'ε_0' is the dielectric constant of free space equal to 8.85 pF/m. The second dielectric constant, 'ε_r', is the relative permittivity of the glass substrate and is equal to 1 for air. A theoretical capacitance value of the sensor-1 is 1.33 pF.

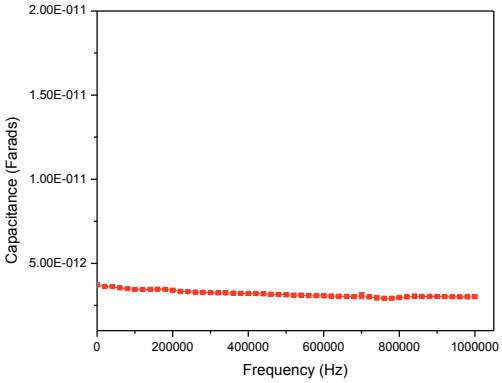


Figure 4. Capacitance of the sensor measured in air.

In this study, HIOKI 3532-50 LCR HiTESTER was used to measure the capacitance value by applying the amplitude of 100 mV in the frequency range of 500 Hz to 1 MHz and is shown in Fig. 4. The average value of sensor capacitance measured is 2.8 ± 0.08pF and thus the sensor is well characterized.

C. *Electrical characterization of sensors in DI-water*

The developed sensor was electrically characterized by measuring electrical impedance spectroscopy at 100mV with the frequency range 1 Hz to 1 MHz in DI-water. Fig. 5a shows the measured bode impedance and phase spectra of interdigitated microelectrode sensor in DI-water along with the fitting spectra and Fig. 5b shows assumed electrical equivalent circuit for fitting.

By analyzing the typical impedance response of the sensors, there are three regions in the impedance spectrum. They correspond to three major electrical components; the double layer capacitance C_{dl}, the solution electrolyte resistance R_s, and the solution dielectric capacitance C_{di}. At the low frequency range, C_{dl} determines the signal. Phenomena occurring in the neighborhood of the electrode are measured in this region, thus surface related process influence this capacitance. At intermediate frequencies, R_s governs the signal, and the conduction of ions in the solution determines the signal. At the high frequency end, the dielectric behavior can be observed and dielectric behavior of solution C_{di} dominates the signal in this region.

To validate equivalent circuit, 52 points of the measured data on the impedance spectrum were automatically selected by the IM-6/THALES software and were used as an input to the equivalent circuit, generating a fitting impedance spectrum. Using this simulation, the values of C_{dl}, R_s, and C_{di} were 3.35 nF, 133.8 kΩ, and 20.9 pF, respectively, with the modulus impedance mean error of 0.6%.

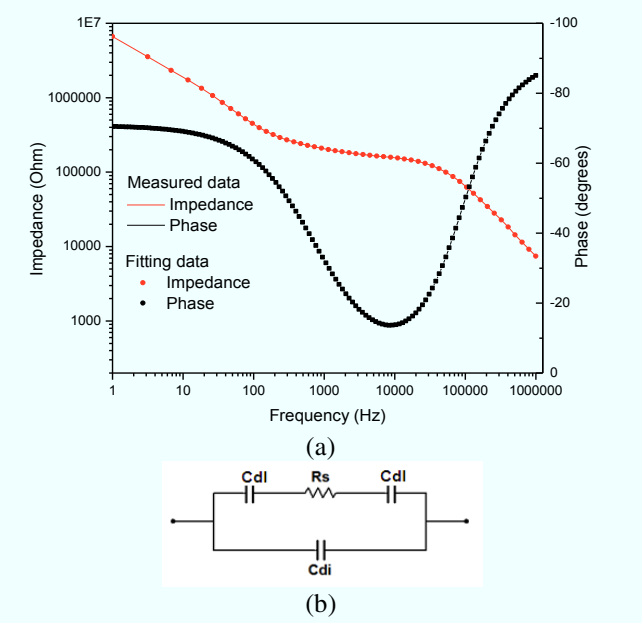


Figure 5. (a) Impedance and phase spectra of sensor in DI water together with their fitting spectra and (b) equivalent circuit.

D. *Detection of E.coli in DI-water*

Developed sensor was used to measure the impedance response of *E.coli* cells in DI-water over a wide range of frequencies. Fig. 6 shows the Bode impedance spectra of *E-coli* cells in DI water with different cell concentrations from $7.1x10^1$ to $7.1x10^7$ cells/ml using interdigitated microelectrode sensor. It can be seen that the impedance spectra of *E-coli* cells in DI water respond significantly different with cell concentrations in the frequency range from 1 kHz to 100 kHz, impedance decreased with the increasing cell concentrations. When bacterial concentration decreased from 7.1×10^7 to 7.1×10^4 cells/ml, the impedance of the

suspension significantly increased. When cell concentrations were lower than 7.1×10^4 cells/ml, the impedance values of the suspensions were not significantly different from each other and from DI water.

The observation of decreases in impedance of *E.coli* in DI-water with increasing cell concentrations indicates that cells with high concentrations are more conductive than those with lower concentrations. Electrical nature of bacterial cell surfaces and ion release from bacterial cells altered the conductivity of DI-water suspension [25].

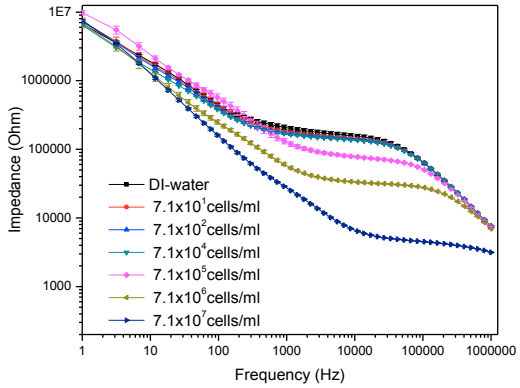


Figure 6. Impedance spectra of *E-coli* cells in water using sensor with the cell concentrations in the range of 7.1×10^1 to 7.1×10^7 cells/ml.

V. Conclusion

An impedance sensor based on interdigitated microelectrode was successfully developed and evaluated for rapid detection of *E.coli* O157:H7 bacteria cells in DI-water. Preliminary testing results indicate that the proposed interdigitated microelectrode sensor has a high potential for quantifying bacterial cells in a label-free, inexpensive, rapid and very simple approach.

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