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Impedimetric method for measuring ultra-low *E. coli* concentrations in human urine

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ABSTRACT

In this study, we developed an interdigitated gold microelectrode-based impedance sensor to detect *Escherichia coli* (*E. coli*) in human urine samples for urinary tract infection (UTI) diagnosis. *E. coli* growth in human urine samples was successfully monitored during a 12-h culture, and the results showed that the maximum relative changes could be measured at 10 Hz. An equivalent electrical circuit model was used for evaluating the variations in impedance characteristics of bacterial growth. The equivalent circuit analysis indicated that the change in impedance values at low frequencies was caused by double layer capacitance due to bacterial attachment and formation of biofilm on electrode surface in urine. A linear relationship between the impedance change and initial *E. coli* concentration was obtained with the coefficient of determination $R^2 > 0.90$ at various growth times of 1, 3, 5, 7, 9 and 12 h in urine. Thus our sensor is capable of detecting a wide range of *E. coli* concentration, 7×10^0 to 7×10^8 cells/ml, in urine samples with high sensitivity.

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1. Introduction

Pathogenic diseases constitute a major cause of death in many developing countries of the world. Current practices for preventing microbial diseases rely upon careful control of various kinds of pathogenic bacteria in clinical medicine, food safety and environmental monitoring. *Escherichia coli* (*E. coli*) O157:H7 is considered to be one of the most dangerous pathogens responsible for the majority of food-borne outbreaks (Alocilja and Radke, 2003; Chemburu et al., 2005) and common healthcare-associated infections such as the urinary tract infection (UTI) (Kunin, 1987; Arredondo et al., 2000). UTIs are common kidney-related diseases in humans, especially women. *E. coli* is responsible for up to 80% of the UTIs (Kunin, 1987; Arredondo et al., 2000), causing up to 70% of community-acquired and 50% of hospital-associated UTIs (Foxman, 2010). Generally, a UTI is defined as the presence of significant bacteriuria ($\geq$10,000 colonies of single bacteria per milliliter urine) and one or more urinary symptoms: hematuria, dysuria, frequency, or urgency (Foxman, 1990). An *E. coli* concentration of $\geq 10^5$ CFU/ml (Hancock et al., 2007; Brzuszkiewicz et al., 2006) in asymptomatic individuals, or *E. coli* concentration of $\geq 10^2$ CFU/ml (Franco, 2005; McIsaac et al., 2007) in symptomatic patients, is typically considered as a UTI. UTIs are rarely fatal (Mach et al., 2011) and although effective treatments are available, the related urinary symptoms are nonspecific and overlap with numerous other non-infectious entities. Thus it is important to monitor the presence of possible pathogens in urine. Conventional bacterial detection method usually involves microbiological culturing and isolation of the pathogen, followed by confirmation with biochemical or serological tests. This method requires labor-intensive procedures and a typical delay of two to three days from urine sample acquisition to delivery of the results (Eisenstadt and Washington, 1996). As a result, so far numerous rapid methods have been developed for UTI diagnosis. The widely used dip-stick method for pathogen detection is based on assessing leukocyte esterase (produced by white blood cells) and nitrite (found in urine in the presence of nitrate-reducing bacteria) (Deville et al., 2004). Recently, urinary flow-cytometry has been developed and widely adopted by centralized laboratories for rapid detection of bacteria, white blood cells, red blood cells, epithelial cells, casts, crystals, yeasts, and spermatozoa (Jolkkonen et al., 2010; Pieretti et al., 2010). But still, these technologies do not provide

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microbiological diagnosis and susceptibility information, which is the keystone of diagnosis, particularly in settings of problematical UTIs (Schwartz and Barone, 2006). Modern clinical microbiology laboratories utilize semi-automated sample processing high-throughput instruments that monitor bacterial CO_2 production for antimicrobial susceptibility testing (Eigner et al., 2005; Thomson et al., 2007), but these systems remain relatively slow. Therefore, easier-to-use and faster systems for pathogen detection in clinical laboratories are desirable.

Recently, interest in electrochemical biosensors for detection of pathogens has increased due to its simple, rapid, label-free, and low-cost detection of biomolecules (Daniels and Pourmand, 2007). Various electrochemical methods such as amperometric (Ruan et al., 2002; Varshney et al., 2005), potentiometric (Ercole et al., 2003), conductometirc (Muhammad-Tahir and Alocilja, 2003) and impedimetric (Varshney and Li, 2008) biosensors have been proposed to detect *E. coli*. Among these electrochemical methods, the impedance technique has been proved to be a promising one for pathogenic bacteria detection due to its portability, rapidity and sensitivity (Katz and Willner, 2003; Ivnitski et al., 1999, 2000; K'Owino and Sadik, 2005). The microbiological impedance methods are the most successful rapid bacterial detection methods. The total impedance during bacterial growth actually consists of two components, medium or electrolyte impedance and electrode/electrolyte interface impedance and these impedances can be measured in different frequency ranges (Hause et al., 1981; Felice et al., 1992, Felice and Valentinuzzi, 1999). Hause et al. (1981) observed that the impedance measured at a low frequency (<100 Hz) was dominated by the interface, while that measured at a high frequency (10,000 Hz) was dominated by the medium effect. However, the frequency range for each component may vary with the difference in the size and dimension of the electrodes. The interface impedance (at low frequencies) will be affected by the attached cells on the electrode surface (Ehret et al., 1997, 1998) and the ionic species generated by bacterial cell growth (Felice and Valentinuzzi, 1999; Yang and Li, 2006; Yang et al., 2004). The medium impedance (at high frequencies) will be affected by the conductance of the medium during bacterial growth (Varshney and Li, 2008). If the cells attach on an electrode surface, they would effectively reduce the electrode area where the current reaches and affect the interface impedance. Bacteria can transform uncharged or weakly charged substrates into highly charged end products, causing an alteration in ionic concentration; this phenomenon would affect the interface impedance and the conductance of the medium.

Although considerable researches have been devoted to the detection of bacteria based on the growth and metabolism in the growth media, the detection of *E. coli* directly in urine samples need to be explained for UTI diagnosis. Thus, the aim of this work is to detect the presence of *E. coli* by monitoring the impedance during the initial bacterial growth in the urine and analyzing the impedance with specific physiological parameters close to the status of the UTI environment. To achieve this target, the impedance technique was incorporated with an interdigitated microelectrode because the interdigitated microelectrode has the ability to sensitively monitor the change of the electrical properties in the immediate neighborhood of its surface and this property will improve the performance of the impedance measurement. In this study, impedance change during the initial growth times of *E. coli* in a UTI environment was measured. The impedance as a function of frequency was analyzed along with an electrical equivalent circuit model to interpret the impedance change caused by the physiological parameters of *E. coli* cells growth.

2. Materials and methods

2.1. Chemicals and reagents

Luria Bertani Miller's media was obtained from AthenaES (Baltimore, MD, United States). Broth was prepared with deionized water from Millipore equipment (Mili-Q water) and sterilized in the autoclave at 121 °C for 1 h. Acetone and isopropanol were purchased from Mallinckrodt (Cambridge, MA, United States).

2.2. Sensor fabrication and experimental setup

The gold interdigitated microelectrode sensor was fabricated in-house on a glass slide (1737, Corning, 36 mm × 15 mm). A standard positive photoresist film S1818 was deposited on the glass substrate and baked on a hot plate at 100 °C for 2 min. An interdigitated microelectrode pattern was transferred onto the substrate by exposing the photoresist to UV light (M-2L mask aligner) and subsequently developing it in MF-319 developer, followed by rinsing in deionized water. A 50-nm gold layer was then sputtered in a high vacuum sputter (Q 150T-ES). To promote the gold adhesion to the glass substrate, a thin layer (2 nm) of chromium had been previously deposited. The lift-off of the unwanted Au/Cr layer and the removal of the undeveloped resist were carried out in acetone with ultra-sonication. Planar gold electrodes were then obtained on the glass substrate with the width and spacing of the finger electrodes of 8 μm. A photograph of an interdigitated microelectrode sensor is shown in Fig. 1a.

Prior to measurements, all interdigitated microelectrode sensors were cleaned with acetone, isopropanol and deionized water, and then were dried with a stream of nitrogen. Polystyrene chambers of 1 ml capacity were bound to the sensor substrate using an adhesive silicon rubber. *E. coli* cells were allowed to grow in this chamber for impedance measurement. The setup is shown in Fig. 1b.

2.3. Bacterial culture and impedance measurement

E. coli cells were grown in Luria Bertani Miller's broth overnight at 37 °C until the exponential phase was reached. The cell concentrations were measured with a hemocytometer under an optical microscope. The culture was then aliquoted into Eppendorf tubes and centrifuged for 10 min at 5000 rpm. After the centrifugation, the supernatant was discarded and the *E. coli* sediment was stored for impedance measurement.

IM-6eX impedance analyzer (Zahner-elektrik CMBH & Co. KG, Germany) was used to measure the impedance response of the biosensor from 1 Hz to 1 MHz. A 100 mV sinusoidal signal is used as the excitation signal to measure the impedance. For the impedance measurement, samples were placed in the polystyrene chamber beneath a glass cover. One contact pad of the interdigitated microelectrode was connected to the test and sense probes, and the other was connected to the reference and counter probes on the IM-6 impedance analyzer. In order to understand the mechanism of impedance change, the measured impedance spectrums were simulated using the equivalent circuit model. To validate the equivalent circuit model, 128 points of the experimental data on the impedance spectrum were selected by the IM-6/THALES software and used as the input data to generate a fitting impedance spectrum.

2.4. Bacterial urine sample preparation

First morning clean-catch urine samples were collected from a healthy female volunteer donor who had given a consent. The urine samples were stored in a refrigerator immediately after

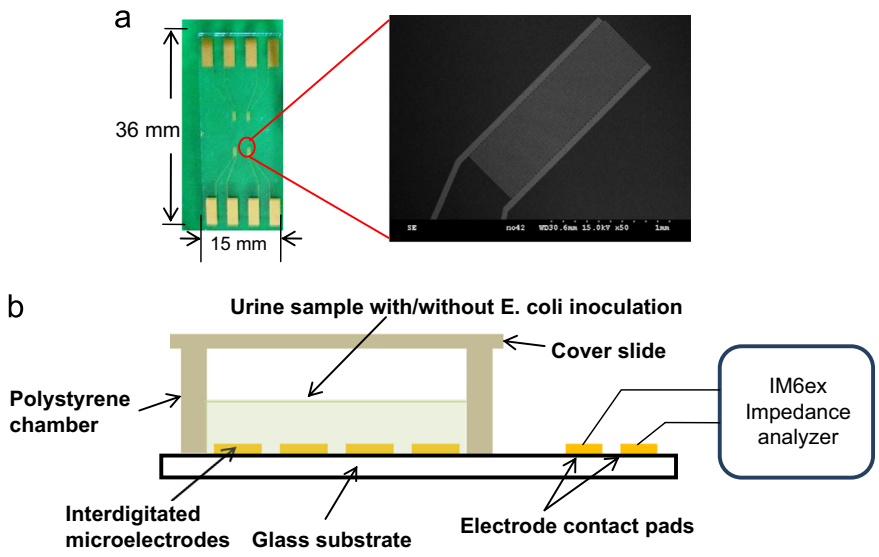

Fig. 1. Material and experimental setup for impedance measurement. (a) Photograph of an interdigitated microelectrode sensor. (b) Cross-sectional view of the interdigitated microelectrode sensor, polystyrene chamber, urine sample during impedance measurement.

collection. Each urine sample was centrifuged in a table-top centrifuge for 15 min at 1500 rpm and the supernatant was used for measurement analysis without any further pretreatment (Ensafi et al., 2011). Aliquots of 1 ml urine were inoculated with *E. coli* to prepare urine samples containing respectively 7×10^0, 7×10^4, 7×10^6, and 7×10^8 cells/ml of *E. coli*. Each *E. coli*-inoculated urine sample was introduced to the impedance chamber and the impedance spectrum was recorded during *E. coli* growth, at 37 °C.

To estimate the *E. coli* concentration in urine at different growth times, *E. coli* cells were grown in test tubes under conditions similar to those in the polystyrene chambers for impedance measurements. At different growth times, urine in the test tubes with *E. coli* was mixed in a vortex mixer and the cell concentrations were measured with the hemocytometer.

3. Results and discussion

3.1. Impedance response of E. coli in urine

Fig. 2 shows the impedance magnitude and phase spectra measured using the interdigitated microelectrode sensor over the frequency range from 1 Hz to 1 MHz at different growth times after inoculating 7×10^4 cells/ml of *E. coli* in urine. The impedance spectra measured with the sensor in the frequency range from 1 Hz to 10 kHz consisted predominately of capacitive reactance, as is evidenced by a phase close to −90°. The impedance spectra above 10 kHz gradually approached pure resistance as the capacitive reactance diminished with the increased frequency. The measurable change with growth time in the impedance magnitude was observed in the low frequency range from 1 Hz to 10 kHz. Thus, the change in the measured impedance value during *E. coli* growth was mainly due to the change in capacitance. This implies that the growth of *E. coli* cells in urine can be monitored by measuring the change in the impedance or the change in the capacitance at frequencies lower than 10 kHz.

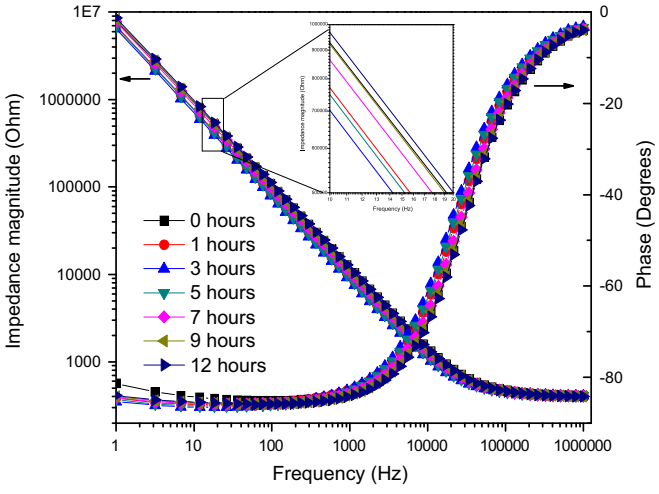

Fig. 2. Bode plot of the impedance spectra of the urine samples inoculated with 7×10^4 cells/ml of *E. coli*, measured on the hour within 12 h.

All the impedance measurement experiments were repeated four times with similar results. Relative standard deviation (RSD) was calculated to determine the repeatability and sensor variability during impedance measurements. In this study, the RSDs of the impedance measurement results were less than 8% ($n=4$).

3.2. Monitoring of E. coli growth in urine

Relative change in impedance at a particular frequency can be used to monitor the activity of the growing bacterial cells (called impedance microbiology). Impedance microbiology is a promising method to measure the changes in medium conductivity caused by bacteria metabolism and also to study the bacterial attachment on solid surfaces, as many pathogenic bacteria attach and colonise to form highly antibiotic resistant biofilms (Varshney and Li, 2008; Parades et al., 2013). Fig. 3a shows the percentage change in impedance at 10 Hz during *E. coli* growth in urine samples with different initial *E. coli* concentrations ranging from 7×10^0 to 7×10^8 cells/ml along with urine samples without *E. coli* inoculation. In order to better understand the effect of initial bacterial cell numbers in growth monitoring, the initial bacterial inoculation time (0 h) data was taken as the reference value for establishing a base line (Parades et al., 2013).

As shown in Fig. 3a, the impedances of the urine samples without *E. coli* inoculation became lower than those of the *E. coli* inoculated urine samples after the growth started. The urine impedance decreased steeply in the first hour and still kept decreasing, although more gradually, at the 12th hour. We had expected that the urine samples without E. coli inoculations

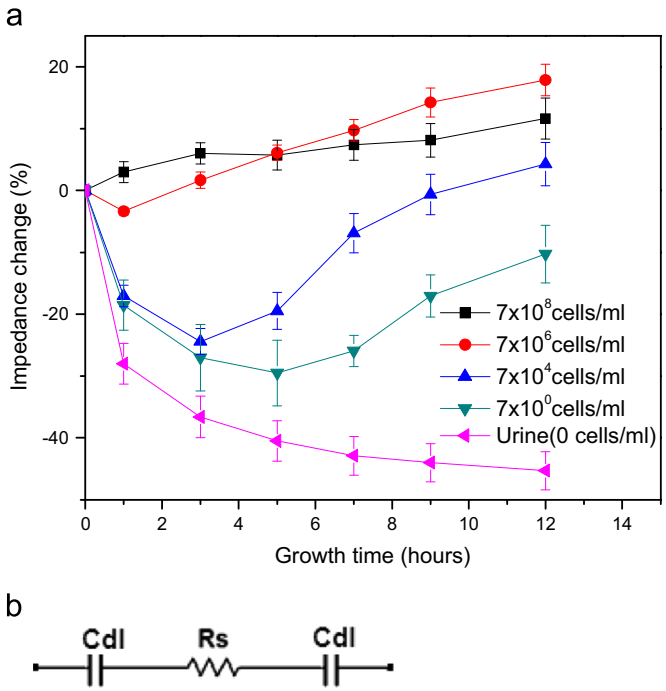

Fig. 3. (a) Time course of the impedance change at 10 Hz within the first 12 h, the initial *E. coli* concentration ranging from 7×10^0 to 7×10^8 cells/ml. (b) Equivalent circuit model for the measured impedance.

impedance will be constant over time; conversely we observed the urine impedance change over time. Urine is chemically complex and the activities of its ions will change over the time interval between voiding and the measurement of impedance. For example, voided urine composition could have reduced in glucose from glycolysis and decreased ketones from volatilization (Fischbach and Dunning, 2009). Therefore, it is possible that the impedance of urine may change over time as a result of its changing chemical composition (Roberts, 1980).

As shown in Fig. 3a, the impedance measured in the urine samples initially inoculated with 7×10^8 cells/ml of *E. coli* gradually increased in the first 12 h. The impedance measured in the 7×10^6 cells/ml samples slightly decreased in the first hour and then kept increasing in the following 11 h. This increasing trend of the impedance measured in the urine samples of these two concentrations was assumed to predominantly arise from the attached bacteria and bacteria-associated materials which obstructed the double-layer charging by blocking the electrode surface (Parades et al., 2013; Muñoz-Berbel et al., 2007). For low *E. coli* concentrations of 7×10^0 and 7×10^4 cells/ml, decreasing of impedance was observed from the beginning to the 5th and 3rd hour, respectively, followed by increasing of impedance until the 12th hour. This behavior follows the growth kinetic profile of a bacterial culture (Varshney and Li, 2008): Initially, a lag phase can be observed with low initial bacterial concentrations. This is the time taken by the bacteria to adapt to the new environment. Initial decreasing trend in impedance for *E. coli* concentrations of 7×10^0 and 7×10^4 cells/ml is related to *E. coli* growth in the lag phase. The decrease in urine impedance is mainly attributed to the change in urine composition (Varshney and Li, 2008; Yang and Li, 2006). In the lag phase of 7×10^0 and 7×10^4 cells/ml cell concentrations, *E. coli* growth could have affected the urine composition; this caused the decreasing trend of the measured impedance to be slower than that in the samples without *E. coli* inoculation. After the lag phase, the bacteria move to the logarithmic growth phase, this involves an exponential increase in the number of cells. This effect is observed by an increasing trend of impedance after the 5th and 3rd hour in low *E. coli* concentrations of 7×10^0 and 7×10^4 cells/ml, respectively. This increasing trend of impedance is believed to be due to the coverage of *E. coli* cells on the electrode surface (Parades et al., 2013; Muñoz-Berbel et al., 2007).

Different trends in impedance change were observed in urine inoculated with low and high concentrations of *E. coli.* Bacterial growth in urine might have caused the change in impedance with respect to growth time. Detailed reasons accounting for the change in impedance behavior due to *E. coli* growth in urine was evaluated by simulating the measured impedance spectrum with an equivalent electrical circuit model. These electrical circuit elements are associated with different physical effects (Gómez et al., 2002). Different electrical models are described in the literature to represent these phenomena (Varshney and Li, 2009). In this work, an electrical circuit model presented by Varshney and Li (2008) as shown in Fig. 3b was used to analyze the variation in the impedance caused by the bacterial growth. The equivalent circuit consisted of two identical capacitors (C_{dl}) connected in series with a resistor (R_s). C_{dl} is the double layer capacitance of the microelectrodes, and R_s represents the bulk electric resistance of the medium.

3.3. Equivalent circuit analysis for impedance measurement system

In order to analyze the reason for the impedance change due to *E. coli* growth in urine, the measured impedance spectra were simulated using an equivalent circuit model $C_{dl} - R_s - C_{dl}$. In this study, the measured impedance spectrum (Fig. 2) can be divided into three regions: a capacitive region in the frequency range from 1 Hz to 10 kHz, a transitional region (partly capacitive and partly resistive) from 10 kHz to 100 kHz and a resistive region beyond 100 kHz. The equivalent circuit model $C_{dl} - R_s - C_{dl}$ conforms to this spectrum and hence it is suitable for analyzing the impedance behavior in this study.

The measured impedance data at various *E. coli* growth time intervals were curve fitted using the equivalent circuit $C_{dl} - R_s - C_{dl}$. A constant R_s value was obtained for every impedance measurement during *E. coli* growth and this phenomenon indicates that the medium resistance of the system was kept constant regardless of bacterial growth. So, we considered only C_{dl} for further analysis.

The percentage change in the measured total impedance (Z) and simulated double-layer capacitance (C_{dl}) is shown in Table 1. Comparing the values of Z and C_{dl}, it can be found that they are almostly inversely proportional. This result is consistent with the theoretical formula $Z = 1/2fC_{dl}$. This behavior indicates that the impedance measured at 10 Hz was almost contributed by the double-layer capacitance. The double layer capacitance can be expressed by the following equation;

$$C_{dl} = \frac{\varepsilon_0 \varepsilon_r A}{d} \quad (1)$$

where ε_0 is the permittivity of free space, ε_r is the effective relative permittivity of the layer separating the ionic charges and the electrode, A is the electrode area, and d is the thickness of the double layer.

As shown in Table 1, different trends in C_{dl} change were observed for various initial *E. coli* concentrations. For 7×10^0 cells/ml, C_{dl} change was 25.65% at the 1st hour and increased to 49.15% at the 5th hour. After 5 h, it gradually decreased to 16.17% at the 12th hour. For 7×10^4 cells/ml, the C_{dl} change was 22.91% at the 1st hour, increased to 37.4% at the 3rd hour and then gradually decreased to -0.66% at the 12th hour. For 7×10^6 cells/ml, the C_{dl} change was 4.19% at the 1st hour and then gradually decreased to -12.93% at the 12th hour. Whereas, for 7×10^8 cells/ml, C_{dl} change

Table 1
Percentage change in impedance and double-layer capacitance at different growth times at various cell concentrations.

Initial *E. coli* concentration, cells/ml	Impedance (Z) & double-layer capacitance (C_{dl})	Percentage change at different growth times with respect to the initial value at 0 hour (%)					
		1 h	3 h	5 h	7 h	9 h	12 h
7×10^0	Z	−18.54	−27.01	−29.5	−25.93	−17.05	−10.26
	C_{dl}	25.65	42.38	49.15	40.81	24.99	16.17
7×10^4	Z	−17.03	−24.41	−19.46	−6.9	−0.63	4.26
	C_{dl}	22.91	37.4	28.6	10.28	3.76	−0.66
7×10^6	Z	−3.37	1.68	6.13	9.73	14.24	17.88
	C_{dl}	4.19	−1.15	−4.61	−7.3	−10.51	−12.93
7×10^8	Z	2.98	6.02	5.74	7.39	8.14	11.66
	C_{dl}	−2.08	−5.92	−5.74	−6.39	−8.04	−10.06

gradually decreased from the initial time of *E. coli* inoculation and attained −10.06% at the 12th hour. The increasing trend of C_{dl} stays longer for lower initial *E. coli* concentrations.

According to the nature of electrode surface contribution to the double-layer element, double-layer capacitance can be used to analyze the bacterial growth in planktonic or form biofilms that adhere to the solid surface depending on their behavior in growth medium. From the result of C_{dl} analysis in Table 1, the initial increase in C_{dl} value of the low concentrations could indicate the planktonic growth that can be simply considered as units that convert uncharged particles into smaller and highly charged molecules. Before the growth of bacteria, the growth medium contains uncharged or weekly charged substances and during bacterial growth these compounds will be converted into highly charged molecules. This metabolism increases the number of polar molecules and small molecules in the double layer, which enhances the dielectric permittivity and decreases the thickness of the double layer. From Eq. (1), these changes in combination result in an increase in the double layer capacitance (Varshney and Li, 2008; Yang and Li, 2006). Decreasing trend in C_{dl} value was observed followed by the increasing trends. The reason for C_{dl} decrease might have been caused by biofilm growth. *E. coli* has the ability to form extra- and intracellular biofilm-like communities within the bladder (Blango and Mulvey, 2010). The osmoprotectant property of glycine betadine and proline betaine increases the ability of *E. coli* and other enteric bacteria to grow in urine (Chambers and Kunin, 1985). In this study, growth medium is urine and hence bacterial biofilm would be formed on the sensor surface. In biofilm growth, the bacterial cells will attach onto the solid surfaces as bacterial community. When environmental

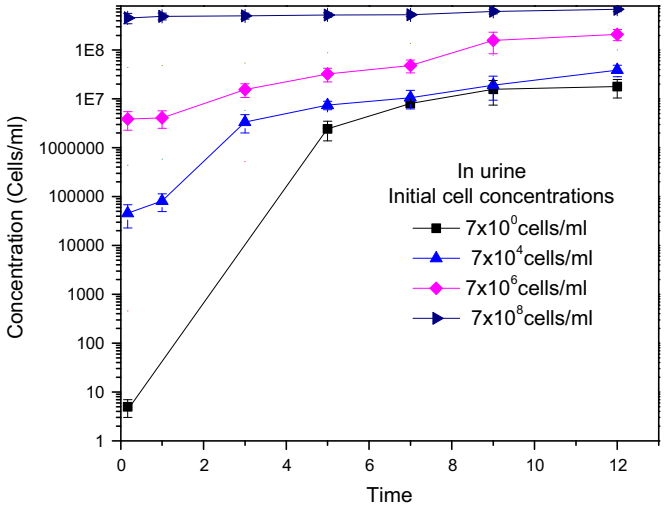

Fig. 4. Time course of the estimated *E. coli* concentration, the initial *E. coli* concentration ranging from 7×10^0 to 7×10^8 cells/ml.

conditions get worse (lack of nutrients and oxygen, excess of toxins, etc.), microorganisms changes their metabolism to produce polymers, which enabled them to adhere and build a favorable structure, such as biofilms (Davies and Geesey, 1995). Therefore, the attachment of bacteria and biofilm onto the electrode surface will decrease the active area of electrode and results in a decrease in the double-layer capacitance (Muñoz-Berbel et al., 2007). This biofilm growth behavior of *E. coli* can be seen from the scanning electron microscopy (SEM) images (Fig. S1, Supplementary material). SEM images depict the cluster of *E. coli* cells and a tight, compact globule of biofilm structures on electrode surface. The number of *E. coli* cells, clusters and biofilm on the electrode surface was gradually increased along the growth times.

Based on the values of C_{dl} change with high initial cell concentrations (7×10^6 and 7×10^8 cells/ml) from Table 1, stationary phase was attained in short growth times. With increasing bacterial cell concentrations, the number of cells attached onto the electrode surface increase. These attached cells caused decrease in C_{dl} and this phenomenon compensated the C_{dl} increase caused by urine composition change along growth time. Therefore, the slight decrease in the double-layer capacitance of high initial bacterial concentration (7×10^8 cells/ml) was due to high cell number in bacterial culture. This result can be as shown in Fig. 4. Fig. 4 shows the *E. coli* concentration in urine with different initial *E. coli* concentrations ranging from 7×10^0 to 7×10^8 cells/ml. As can be seen from Fig. 4, the *E. coli* concentration increased exponentially from the time of inoculation to the growth time of 12 h in low initial cell concentrations of 7×10^0 and 7×10^4 cells/ml and stationary phase of cell growth was observed in high initial cell concentrations almost from the time of inoculation.

In this study, impedance measurements were conducted in urine samples, which should contain proteins. The size of the urinary proteins are mostly in nanometer level (Erickson 2009; Thongboonkerd et al., 2002), whereas the size of *E. coli* is approximately 1×3 μm (Reshes et al., 2008). The size of *E. coli* is bigger than that of the urinary proteins and also the number of *E. coli* cells in urine sample increased with time as shown in SEM pictures (Fig. S1, Supplementary material). This result reveals that the change in impedance observed in our system during *E. coli* growth in urine was mainly contributed by the *E. coli* and not by the proteins. Hence the proteins present in urine did not influence the results of impedance measurements.

3.4. Detection of E. coli in urine samples

The performance of the proposed impedimetric interdigitated microelectrode sensor can be demonstrated at various growth times in connection with the impedance monitoring of the growth of different concentrations of *E. coli*. From Fig. 3a, the change in impedance values (at 10 Hz) due to *E. coli* growth at various growth times was plotted. Fig. 5 shows a

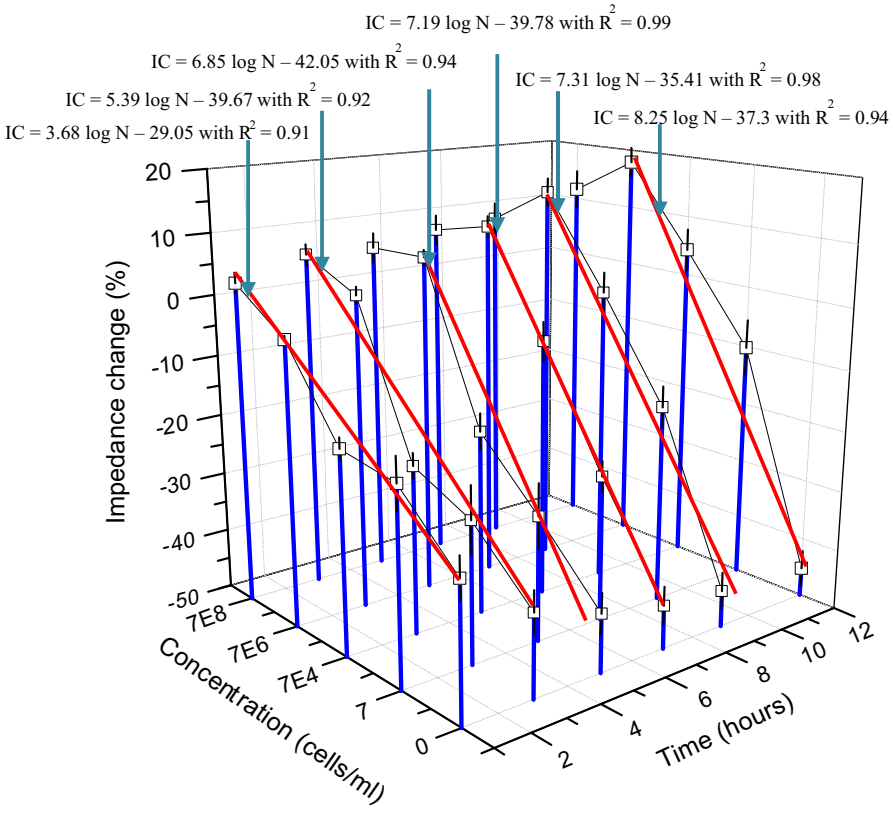

Fig. 5. Time-graded. linear regression lines of the measured impedance and the initial *E. coli* concentration.

three-dimensional representation of the changes in impedance as a function of initial cell concentration and growth time. It can be seen that the impedance change significantly increase with the increase of the bacterial concentrations. A linear relation between impedance change and concentration was obtained at each growth time. Linear increase of the impedance change was observed between 0 and 7×10^8 cells/ml of bacterial concentrations at 1 h and 3 h. At 5, 7, 9, and 12 h of growth times, linear increase of the impedance change was observed from 0 to 7×10^6 cells/ml of bacterial concentrations, followed by a little decrease at 7×10^8 cells/ml. The increase in impedance change was caused by the attachment and biofilm formation on electrode surface; Decrease in impedance change at 5, 7, 9, 12 h of growth times was observed because of the saturation state in 7×10^8 cells/ml. Linear relationship between the impedance change (IC, %) and the initial cell concentration (N, cells/ml) in urine was found at all growth times (1, 3, 5, 7, 9 and 12 h) with the coefficient of determination, $R^2 > 0.90$. Slope value increased from 3.68 at 1 h to 8.25 at 12 h of growth time. Increase in slope value indicates that our sensor sensitivity increases with increasing growth times. The threshold of UTI for asymptomatic individuals is 10^5 CFU/ml (Hancock et al., 2007; Brzuszkiewicz et al., 2006) and for symptomatic patients is 10^2 CFU/ml (Franco, 2005; McIsaac et al., 2007), and our proposed device is sensitive in detecting such low concentration.

Recently, many interdigitated gold microelectrode-based impedance sensors for detecting bacteria including *E. coli* have been reported (Dweik et al., 2012; Kim et al., 2012; Laczka et al., 2008; Paredes et al., 2014). Dweik et al. and Laczka et al. developed an antibody immobilized interdigitated gold microelectrodes based impedance sensing platform for the detection of *E. coli*. The detection limits of these sensors are 2.5×10^4 CFU/ml (Dweik et al., 2012), 1.5×10^3 cells/ml (Laczka et al., 2008) and these devices are not sensitive in detecting very low *E. coli* concentrations. Our proposed label-free impedance monitoring device can detect very low concentration of *E. coli* (7 cells/ml) without electrode modification. Paredes et al. and Kim et al. proposed a label-free interdigitated gold microelectrodes impedance sensor for real-time characterization of the microbiological biofilms growth behavior as a way to detect the bacterial cells. They monitored the *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* biofilms growth in culture media prepared through a laboratory protocol. But none of these sensors has been used in a real sample such as urine. The interdigitated gold microelectrode sensor proposed in this study was successfully used to monitor the *E. coli* biofilm growth behavior in a real sample (human urine) and this method can effectively detect *E. coli* at a concentration as low as 7 cells/ml without modifying the sensor surface. This is the highlight of this work and thus the interdigitated gold microelectrode sensor reported herein is distinct from the previously reported interdigitated gold microelectrode sensors.

Table S1 (Supplementary material) shows the comparison of our proposed study with other detection methods for UTI diagnosis. In healthcare unit, urinalysis conducted with dipstick strips is an on-site UTI screening method. It reveals the presence of leukocytes, red blood cells and nitrites in urine, but positive results need to be confirmed by culture method. Conventional culture technique is labor-intensive, time-consuming and expensive. Sample collection and pre-treatment has to be done with care to avoid contamination. Recently, Yang et al. (2011) developed a lab-on-a-chip that can detect *E. coli* in simulated urine samples with the lowest detection limit of 3.4×10^4 CFU/ml, which is not sensitive enough to detect very low concentrations of *E. coli*. Nevertheless, the impedance-based sensor proposed in this study can detect *E. coli* in urine samples of as low as 7 cells/ml within 12 h without sample pre-treatment.

4. Conclusion

In this study, interdigitated microelectrode sensors were successfully used for quantitative detection of *E. coli* in urine by label-free impedance spectroscopy. Change in impedance due to *E. coli* growth in urine was observed at low frequencies (< 10 kHz). To interpret the change in impedance during *E. coli* growth, an equivalent electrical circuit model consisting of two double-layer capacitors and a medium resistor was introduced and the equivalent circuit analysis indicates that the change in impedance at low frequencies was caused by the double-layer capacitance. The double-layer capacitance relates to the coverage of *E. coli* on the electrode surface by attachment and biofilm formation during *E. coli* growth. The change in double-layer capacitance showed dependence on both the growth time and the concentration of *E. coli*. This equivalent circuit analysis indicates the low-frequency impedance was the key parameter for the detection of *E. coli* in urine. A linear relationship between the impedance change at 10 Hz and the initial *E. coli* concentration in urine was found at all growth times (1, 3, 5, 7, 9 and 12 h) with the coefficient of determination, $R^2 > 0.90$. Our result highlights the possible *E. coli* detection in urine with a large detection range at different growth times. Also our results reveal that the impedance change due to *E. coli* growth in urine was not affected by various human proteins present in the urine. The bacterial growth monitoring approach for *E. coli* detection is highly promising for 1 cell/ml in a test sample. Therefore, the interdigitated microelectrode impedance sensor has been proven to be an effective tool for monitoring *E. coli* growth and quantifying *E. coli* in urine for UTI diagnosis. This experimental setup could be easily embedded into urinary catheters and other microbial techniques that are routinely used in clinical and laboratory tests for UTI diagnosis.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.11.027.

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