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Research review paper

Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria

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Abstract

The realization of rapid, sensitive, and specific methods to detect foodborne pathogenic bacteria is central to implementing effective practice to ensure food safety and security. As a principle of transduction, the impedance technique has been applied in the field of microbiology as a means to detect and/or quantify foodborne pathogenic bacteria. The integration of impedance with biological recognition technology for detection of bacteria has led to the development of impedance biosensors that are finding wide-spread use in the recent years. This paper reviews the progress and applications of impedance microbiology for foodborne pathogenic bacteria detection, particularly the new aspects that have been added to this subject in the past few years, including the use of interdigitated microelectrodes, the development of chip-based impedance microbiology, and the use of equivalent circuits for analysis of the impedance systems. This paper also reviews the significant developments of impedance biosensors for bacteria detection in the past 5 years, focusing on microfabricated microelectrodes-based and microfluidic-based Faradaic electrochemical impedance biosensors, non-Faradaic impedance biosensors, and the integration of impedance biosensors with other techniques such as dielectrophoresis and electropermeabilization.
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Keywords: Impedance; Bacteria detection; Biosensor; Microfluidic chip; Microelectrodes

Contents

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1. Introduction

1.1. Problems with foodborne pathogens and diseases

Diseases caused by foodborne pathogens have been a serious threat to public health and food safety for decades and remain one of the major concerns of our society. Today, there are more than 250 known diseases caused by different foodborne pathogenic microorganisms, including pathogenic viruses, bacteria, fungi, parasites, marine phytoplankton, and cyanobacteria, etc. Among these, bacteria are the most common foodborne pathogens, accounting for 91% of the total outbreaks of foodborne illness in the USA (Beran et al., 1991; Potter et al., 1997). Foodborne pathogens cause an estimated 76 million illnesses, accounting for 325,000 hospitalizations and more than 5000 deaths in the United States each year (Mead et al., 1999). Besides, foodborne diseases are extremely costly. The U.S. Department of Agriculture (USDA) Economic Research Service (ERS) estimates that the medical costs and productivity losses associated with five major pathogens *E. coli* O157:H7, non-O157 STEC (Shiga Toxin-Producing Escherichia coli), *Salmonella* (non-typhoidal serotypes only), *Listeria monocytogenes* and *Campylobacter,* is at least $6.9 billion annually (USDA/ERS, 2002).

Continuous outbreaks of illness and recalls of food products due to foodborne pathogens have caused alarm in the Federal Government and pressures on regulatory and inspection agencies. Beginning early in the 1900s with the creation of the earliest regulatory programs to the creation of the President's Council on Food Safety in 1998, the Federal Government has endeavored to improve the safety of the American food supply in a variety of ways. Currently, the Food Safety and Inspection Service (FSIS) within the USDA, the Food and Drug Administration (FDA) within the Department of Health and Human Service (HHS), and the Environmental Protection Agency (EPA) are the three agencies at the Federal level that are taking major responsibilities for regulating food safety and developing novel inspection methods. In addition, the Institute of Food Technologists (IFT) has implemented the Hazard Analysis Critical Control Point (HACCP) approach as a major step in the present and future of food safety nationally and internationally (Stier, 1993). The issue of foodborne pathogens has also captured the attention and concern of the scientific community, food industry, academia, and the public. The public has become increasingly aware and concerned about the health risks posed by these foodborne pathogens.

1.2. Overview of detection of foodborne pathogens

Monitoring is the first control point in the prevention of diseases caused by foodborne pathogens. Effective detection and inspection methods are necessary to control pathogens in food products. Conventional microbiological methods have been a standard practice for the detection and identification of pathogens in food for nearly one century and continue to be a reliable standard for ensuring food safety. These conventional methods rely almost exclusively on the use of specific agar media to isolate and enumerate viable bacterial cells in samples. The procedure of such a method usually includes microbiological culturing and isolation of the pathogen, followed by confirmation by biochemical and/or serological tests, taking up to 5–7 days to get a confirmed result for a particular pathogenic organism (Swaminathan and Feng, 1994; Vasavada, 1997). While reliable, these conventional methods are time consuming and labor intensive, and are therefore not suitable for modern food quality assurance to make a timely response to possible risks. As a result, over the past 25 years, numerous rapid methods have been developed to reduce the assay time. Approaches that have been studied or are currently being studied include miniaturized biochemical tests, physicochemical methods that measure bacterial metabolites, highly specific nucleic acid-based tests, antibody-based methods, and some fully automated instrumental diagnostic systems (Swaminathan and Feng, 1994; Silley, 1994). Many of these rapid methods developed in earlier years have been extensively reviewed by Swaminathan and Feng (1994), Van Der Zee and Huis in't Veld (1997), Vasavada (1997), Ivnitsk et al. (1999) and Hall (2002). To date, well studied rapid methods such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) have reduced the assay time to 10–24 h and 4–6 h, respectively, and have achieved detection limits varying from 10^1 to 10^6 cfu/ml (cfu = colony forming units). Recently, various biosensors have been developed for pathogenic bacteria detection because of their improved speed, sensitivity and reliability. Sensitivity of a conventional biosensor is between 10^3 and 10^4 cfu/ml with an assay time around 2 h under ideal conditions (Ivnitsk et al., 1999, 2000; Rand et al., 2002; Su and Li, 2004).

Despite the significant reduction in assay time and improvement in detection limit of these rapid methods, there are still key issues and concerns that need to be considered in the development of rapid methods for detection of foodborne pathogens. Amongst these issues are: the number of samples per operation

(high throughput format), differentiation of live and dead cells (since dead cells are usually not pathogenic), automation, cost, simplicity, training, and accuracy. Ideally, a useful rapid method is expected to be able to detect low cell numbers since the presence of even a single pathogenic organism in the body or food may be an infectious dose. For example, the infectious dosage of pathogens such as *E. coli* O157:H7 and *Salmonella* is as low as 10 cells; and the existing coliform standard for *E. coli* in water is 4 cells/100 ml (Federal Register 1990, 1991; Greenberg et al., 1992). For many food samples, the demand for detection limit is less than 1 cell per 25 g of food. To date, an ideal rapid and automated system that meets all the needs mentioned above does not exist. The current strategy for developing a rapid method is to choose and combine some functions that can meet specific applications. Generally, low cell numbers take a long time to detect, therefore, sometimes, a compromise or trade-off between detection limit and detection time has to be considered.

1.3. Role of impedance techniques for bacteria detection

As a principle of transduction, the impedance technique has been applied in the field of microbiology as a means to detect and/or quantify bacteria. One common impedance method for detection of bacterial growth is *impedance microbiology*. It is based on the measurement of changes in electrical impedance of a culture medium or a reaction solution resulting from the bacterial growth. This growth-based impedance technique allows us to distinguish between viable and dead cells. Such a method has been developed as a rapid method that can detect bacteria within 24 h. In 1992, the impedance method was approved by the Association of Official Analytical Chemists International (AOAC) as a first action method for screening *Salmonella* in food samples (Gibson et al., 1992; AOAC, 1996). Most applications of the impedance technique for detection of bacteria were reviewed by Silley and Forsythe (1996) and Wawerla et al. (1999).

In the past few years, some new aspects have been added to this subject which have made the impedance technique a more valuable technique for studies of bacterial growth detection. These new aspects include the use of different electrode systems and the analysis of impedance components using equivalent circuits for better improvement the detection systems (Yang et al., 2003, 2004a; Yang and Li, 2006). The advances in microfabrication technologies have launched the use of microfabricated microarray electrodes in impedance detection and the miniaturization of impedance microbiology into a chip format, which has shown great promising in rapid detection of bacterial growth (Gomez et al., 2001, 2002, 2005).

The integration of impedance technique with biosensor technology has led to the recent development of impedance biosensors that is expending rapidly for bacteria detection (Ruan et al., 2002; Yang et al., 2004b; Radke and Alocilja 2005). Impedance biosensors for bacteria detection are based on impedance analysis of the electrical properties of bacterial cells when they are attached to or associated with the electrodes. The impedance biosensor methods have substantially reduced the assay time down to between 30 min and 2 h compared with growth-based impedance methods. Particularly, significant progress has been made in developing detection platforms which exploit recent advances in microfabrication and electromechanical nanotechnology. The dimensional compatibility of these microfabricated biosensors with the target bacterial cells has enabled them to detect the binding of bacterial cells on their surfaces without any amplification step.

This paper reviews the progress and applications of impedance microbiology for bacteria detection, particularly recent development in this field, including the use of interdigitated microelectrodes, chip-based impedance microbiology, and the use of equivalent circuits for analysis of the impedance system. The paper also reviews the significant developments of impedance biosensors for bacteria detection in the past 5 years, focusing on microfabricated microelectrodes-based and microfluidic-based Faradaic electrochemical impedance biosensors, non-Faradaic impedance biosensors, and the integration of impedance biosensors with other techniques.

2. Impedance microbiology

2.1. Basis of impedance microbiology

In impedance microbiology, the impedance change is typically measured using a pair of electrodes submerged in the growth medium or the reactant solution. The measurement can be performed in two ways, direct or indirect measurement (Silley and Forsythe, 1996). In the direct technique, a pair of metal electrodes is immersed in the medium that is inoculated with the testing bacteria. The impedance change caused by bacterial metabolism in the medium is monitored with time. The impedance change in the medium is mainly produced by the release of ionic metabolites from live cells. There are two main origins of ion release by bacteria into their growth environment (Owicki and Parce, 1992). One is energy metabolism (catabolism) in which bacteria consumes oxygen and sugars and produces carbon dioxide and organic acids. For example, the conversion of a non-ionized glucose to two molecules of lactic acid will increase the conductivity of the culture medium. Further metabolism will take the lactic acid and three oxygen molecules to carbonic acid. The smaller and more mobile bicarbonate ion is a more effective ionic conductor than the lactate ion (Don Whitley Scientific, 1999). Hydrogen ions are nearly seven times more effective as an ionic conductor than sodium ions (Eden & Eden, 1984). The other origin is ion exchange through the cell membrane. Ions (such as K^+ and Na^+) are actively transported across ion channels embedded in the cell membrane, which serves to regulate the membrane potential and the osmotic difference between the interior and exterior of the cells. While the energy metabolism is the major origin of the ion release from cells to the environment, the ion exchange process is a small contribution to the ion release from cells. What is clear, is that these ion release processes cause changes in the ionic composition of the medium and consequent changes in the conductivity of the medium, which is the basis for the measurement of relative impedance changes. Unlike the direct

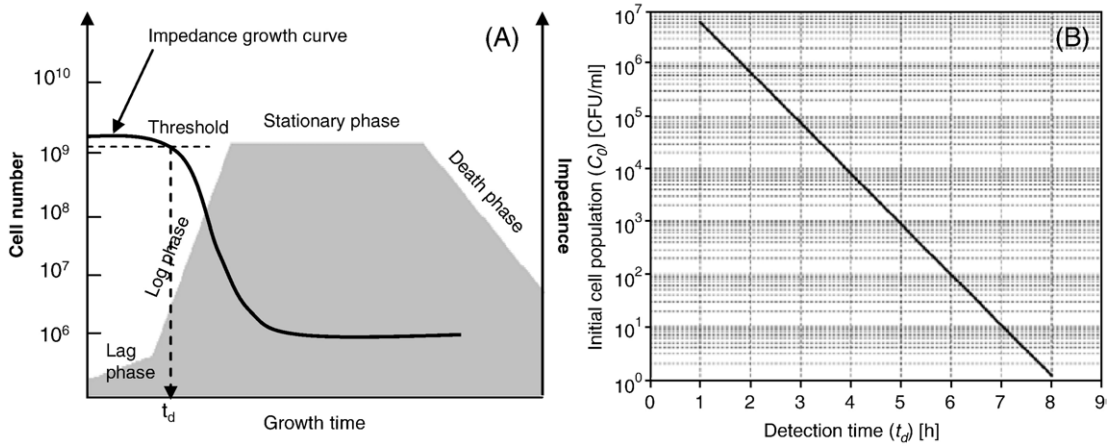

Fig. 1. (A) A generalized impedance growth curve with the threshold and the detection time, along with the typical bacterial growth phases of a bacterial growth curve. (B) the relationship between the detection time and the initial cell concentration (Reprinted with permission from Sensors and Actuators B and kind permission from Gomez et al., 2002).

technique, the indirect technique does not directly measure the impedance change in the bacterial growth medium. Instead, the electrodes are immersed in a separate solution (usually a potassium hydroxide solution) instead of the inoculated growth medium. The gases (mainly CO_2) produced from bacterial metabolism are absorbed by the potassium hydroxide solution, which leads to a decrease in the conductance of the alkaline solution.

To detect bacteria, the impedance systems measure the relative or absolute changes in conductance, capacitance, or impedance, at regular time intervals during the growth of bacteria at a given temperature. The measured electrical signals are then graphically plotted on the ordinate against the incubation times on the abscissa, producing impedance growth curves. A typical impedance growth curve is illustrated in Fig. 1A. The impedance value is quite stable in the first region of the impedance growth curve, and then it starts decreasing. The time corresponding to a point at which the decrease in impedance value exceeds a threshold value is defined as *the detection time,* t_d (Fig. 1A). Generally, the detection time does not appear until the bacteria number reaches approximately 10^6–10^7 cfu/ml (as determined by the plating method). The impedance value finally reaches a plateau, where the bacteria has grown to a high concentration of 10^8 cfu/ml or greater, and all the resources in the medium have been metabolized to end products. The shape of the impedance growth curve matches very well with the typical bacterial growth phases, which includes the lag phase where bacteria metabolize but do not multiply, the log or exponential growth phase where the bacteria multiply exponentially, and the stationary phase where the bacterial cell number remains relatively constant (Talaro, 2005).

Theoretical analysis, consistent with experimental observation, shows that the detection time (t_d) is related to the initial cell concentration C_0. Their relationship can be expressed by Eq. (1).

$$\log(C_0) = -at_d + \beta \quad (1)$$

Where, α ($\alpha > 0$) and β are constants that associate with the certain microorganism, the medium, and growth conditions, etc. Taking the values of $\alpha = 0.96$ and $\beta = 7.75$ reported by Eden and Eden (1984), the plot in Fig. 1B represent the relation expressed in Eq (1), for t_d in hours and C_0 in cfu/ml (Gomez et al., 2002). As shown in Fig. 1B, the detection time of the conventional impedance microbiological methods ranges from about 1 h to 8 h for initial bacterial concentration of 10^7 to 10^1 cfu/ml. Others (Dupont et al., 1996) reported $\alpha = 1.08$ and $\beta = 11.33$ for a particular set of food samples tested for *E. coli*, for C_0 in cfu/100 g food. Detection times of ~ 1 h to ~ 7 h for initial bacterial concentrations of 10^7 and 40 cfu/ml, respectively, were reported by Edmiston and Russell (1998).

2.2. Classic impedance microbiology

Although the first impedance measurement to detect microorganism growth was described one century ago (Stewart, 1899), it did not receive much attention until in the middle of 1970s. Thereafter, an increasing number of papers, including notable work of Ur and Brown (1974, 1975), Cady (1975, 1978), Cady et al. (1978) and the very important work of Eden and Torry Research Station (Richards et al., 1978; Eden and Eden, 1984), were published to promote impedance microbiology as a rapid method for detection of bacteria within 24 h.

Over time, much work has been done in the field of media engineering, since direct impedance microbiology is based on the monitoring of impedance change in the medium. The principles of medium design, fundamental to traditional microbiology, are equally important to impedance microbiology. First, a medium must support the selective growth of the target bacteria, which provides the selectivity to the impedance microbiological methods. Second, the medium must be formulated to provide optimal impedance signals. For instance, one can predict that weakly buffered media would allow a greater conductance change than more strongly buffered media.

The detection of *Salmonella* has been a major focus of impedance microbiology. Numerous papers have been published on the subject of media engineering for *Salmonella* detection with emphasis on the optimization of the electrical signal and the support of selective growth of *Salmonella*. The

initial impedance detection medium for *Salmonella* was selenite–cystine/trimethylamine oxide/dulcitol (Easter and Gibson, 1985). Due to negative results for some *Salmonella* strains, dulcitol in the medium was replaced by mannitol (Gibson, 1987; Ogden and Cann, 1987). Greater impedance changes were obtained with mannitol or deoxyribose in place of dulcitol (Pettipher and Watts, 1989a). Other *Salmonella* impedance medium for selective growth between genus, species, and strain were developed by several research groups (Ogden, 1988; Bullock and Fordsham, 1989; Pettipher and Watts, 1989b; Simth et al., 1990; Davda and Pugh, 1991). Blivet et al. (1998) developed a new impedance medium that supported the growth of *Salmonella* serotypes and inhibited non-*Salmonella* strains in pure culture. The impedance technique was accepted as a first action method for screening *Salmonella* in food by the Association of Analytical Communities International (AOAC) in 1992 (Gibson et al., 1992), and was approved as a final action method for the detection of *Salmonella* in food by AOAC in 1996 (AOAC, 1996).

Indirect impedance methods sometimes are cheaper and simpler than direct impedance methods because they do not require specially formulated impedance media. Such an approach has been reported for the rapid detection of *Salmonella* (Madden et al., 1996, Blivet et al., 1998).

The impedance microbiological methods are perhaps the most successful of all the recently introduced rapid methods in automation. Several commercial analytical instruments are based on the principles of classic impedance microbiology. These systems include Bactometer (Bio Merieux, Nuertingen, Germany), the Malthus systems (Malthus Instruments Ltd., Crawley, UK), rapid automated bacterial impedance technique (RABIT) (Don Whitley Scientific Ltd., Shipley, UK), and BacTrac (Sy-Lab, Purkersdorf, Austria).

Much work has been done to apply the impedance technique to the detection of different bacteria in different samples using these existing commercial instruments. For example, the Bactometer system was used to detect total microbial load in meat and fish as reported by Russell et al. (1994) and Van Spreeken and Stekelenburg (1986), respectively. Detection of *E. coli* in potable water and shellfish was reported by Colquhoun et al. (1995) and Dupont et al. (1996), respectively. Edmiston and Russell (1998) conducted a study to evaluate a conductance method for rapidly enumerating *E. coli* from broiler carcasses using the Bactometer system. Selective detection of *Staphylococcus aureus* with impedance measurement was carried out in a nutrient broth containing 10 ppm of nalidixic acid and 10 ppm of acriflavine (Glassmoyer and Russell, 2001).

Impedance microbiological techniques have also been used to detect many other bacteria species, such as *Enterobacteriaceae*, coliforms, *Listeria* spp., and *L. monocytogenes* in various samples. Impedance techniques can also be used for monitoring the growth behavior of bacteria. The impedance growth curves under different conditions have been found to be characteristics of bacteria species (Fehlhaber and Kruger, 1998). Most applications of impedance microbiology in earlier years have been extensively reviewed by Silley and Forsythe (1996), Valentinuzzi et al. (1996) and Wawerla et al. (1999).

2.3. Impedance-splitting methods

While most impedance microbiological methods measure only the conductance of the medium using a pair of electrodes submerged in the inoculated medium at a fixed frequency, several studies have found that the total impedance during bacterial growth actually consists of two components which can be measured at different frequency ranges. One component contributed by the medium, is known as medium or electrolyte impedance, and the other component contributed by the electrode and the electrode/electrolyte interface, is known as electrode or interface impedance (Hause et al., 1981; Felice et al., 1992, Felice and Valentinuzzi, 1999). These two contributing components can be distinguished by changing the frequency. Hause et al. (1981) observed that the electrode impedance dominated at low frequency (<100 Hz), while impedance measured at 10,000 Hz was dominated by the media effect. Felice et al. (1992) investigated medium and interface contributions to the impedance during bacterial growth by using the frequency dependent properties of Warburg's model. They used stainless wire electrodes for both tetrapolar and bipolar impedance measurement at several frequencies between 18 Hz and 18 kHz, by which medium impedance was obtained at high frequencies (>5 kHz) and interface impedance was negligible at such high frequencies.

The identification of electrode and medium impedance components has led to the development of impedance-splitting methods for the detection of bacteria (Pless et al., 1994; Futschik and Pfützner, 1995; Futschik et al., 1995). SY-lab (Neupurkersdorf, Austria) has developed a commercial instrument, BacTrac™, by which impedance measurements are made at 1 kHz for both the electrode impedance (*E*-value) and medium impedance (*M*-value). Rapid detection of *Bacillus stearothermophilus* has been achieved using the impedance-splitting method (Flint and Brooks, 2001).

2.4. Equivalent circuit analysis for impedance components

The medium impedance and the electrode impedance and their frequency dependent contributions to the total impedance can be well interpreted by analyzing the system using an equivalent circuit. From the electric point of view, a simple equivalent circuit of a resistor and a capacitor in series, is sufficient to represent the behavior of the impedance test system when two electrodes are immersed into a conductive medium. To be useful, the elements in an equivalent circuit should always have a basis in the physical electrochemistry of the system. Typically, the impedance between the two electrodes (Fig. 2A) can be represented by a simple series circuit shown in Fig. 2B, which consists of a solution resistor (R_s) between the two electrodes and a double layer capacitor of each electrode (C_{dl}). Yang et al. (2003) demonstrated the feasibility of using the equivalent circuit to analyze the impedance detection system for bacteria growth. They showed that the impedance spectrum obtained in a growth medium with 1.1×10^3 cfu/ml of *Salmonella* typhimurium cells agreed well with the fitting spectrum (Fig. 2C). The agreement of the two spectra verified the

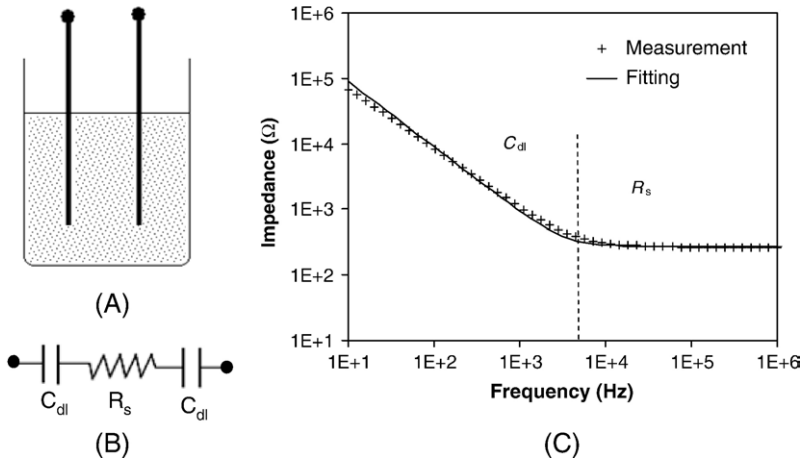

Fig. 2. (A) Typical setup of the two-electrode system for impedance measurement. (B) A simplified equivalent circuit for the two electrode system. C_{dl} and R_s represent the double layer capacitance of the electrode and the solution resistance. (C) Plot of Impedance vs. frequency. R_s is the medium resistive region. C_{dl} is the double layer capacitive region. The cell number of *S. typhimurium* was 1.1×10^3 CFU/ml. Amplitude: 5 mV (Reprinted with permission from Biosensors and Bioelectronics and kind permission from Yang et al., 2003).

equivalent circuit for understanding the impedance change in the system.

Based on the equivalent circuit, when an alternating sinusoidal potential is applied to the system under test, the impedance (Z) of the system is a function of its resistance (R_s), capacitance (C_{dl}) and the applied frequency (f), as expressed in Eq. (2):

$$|Z| = \sqrt{R_s^2 + \left(\frac{1}{\pi f C_{dl}}\right)^2} \tag{2}$$

This equation explains the observation from impedance growth curve that impedance always decreases when bacteria grow in the medium. The decrease in impedance comes from two parts, decrease in R_s and increase in C_{dl}. It is understandable that bacteria metabolize large and uncharged molecules into small and charged molecules in the medium, which results in a decrease in R_s of the medium. The increase in the double layer capacitance is related to the ionic composition change at the immediate neighborhood of the electrode surface which is referred to as the double layer. The value of double layer capacitance depends on many factors including electrode potential, temperature, ionic concentrations, type of ions, and electrode surface properties (e.g. electrode roughness, adsorption, etc.). In this case, the double layer capacitance can be expressed with a simple formula as follows:

$$C_{dl} = \frac{\varepsilon_{dl} A}{d} \tag{3}$$

Where ε_{dl} is the dielectric permittivity of the charged double layer; $\varepsilon_{dl}=\varepsilon_0\varepsilon_\rho$, ε_0 is the permittivity of free space and ε_ρ is the effective dielectric constant of the layer separating the ionic charges and the electrode; A is the electrode area, and d is the thickness of the double layer. Before the growth of bacteria, the medium contains uncharged or weakly charged substrates, such as lactose. Upon the growth, these compounds are transformed into small highly charged ions. As a result, the number of polar molecules and small charged molecules in the double layer increases, which enhances the dielectric permittivity, ε_{dl}, and decreases the thickness of the double layer, d, at the same time. These changes in combination result in an increase in the double layer capacitance and a consequent decrease in impedance.

Eq. (2) also gives the best explanation for the frequency dependent properties of the impedance measurement during bacterial growth. As shown in Fig. 2C, the total impedance decreases linearly with the increasing frequency in the low frequency range from 10 Hz to 10 kHz, while it becomes independent of the frequency in the high frequency range from 10 kHz to 1 MHz. At low frequencies (<10 kHz), since the double layer capacitance offers essentially high impedance, it becomes the main source contributing to the total impedance, such that the medium resistance can be ignored. This region is defined as the double layer capacitive region in which the electrode impedance can be detected (Fig. 2C, C_{dl} region). When in the high frequency range (> 10 kHz), the double layer capacitance almost offers no impedance, and its contribution to the total impedance nears zero. Thus, the only contribution to the total impedance at high frequencies is the medium resistance which is independent of the frequency. This region is defined as the resistive region in which the conduction of ions in the medium dominates the signal (Fig. 2C, R_s region).

Therefore, the changes in the double layer of the electrode and changes in the medium during the growth of bacteria could both be detected by impedance measurements performed at different frequencies. The frequency dependent properties of these two components have been demonstrated in different impedance systems for detection of bacterial growth (Yang et al., 2003, 2004a).

Felice et al. (1999) reported a capacitance method for quantification of bacterial content in milk using two identical stainless steel electrodes and measuring capacitance at 1 kHz. It was found that the interface capacitance presented the maximal growth variations of 150–1250%, which was always larger than those displayed by conductance (36–150%). With this method, shorter detection times and a better coefficient of correlation

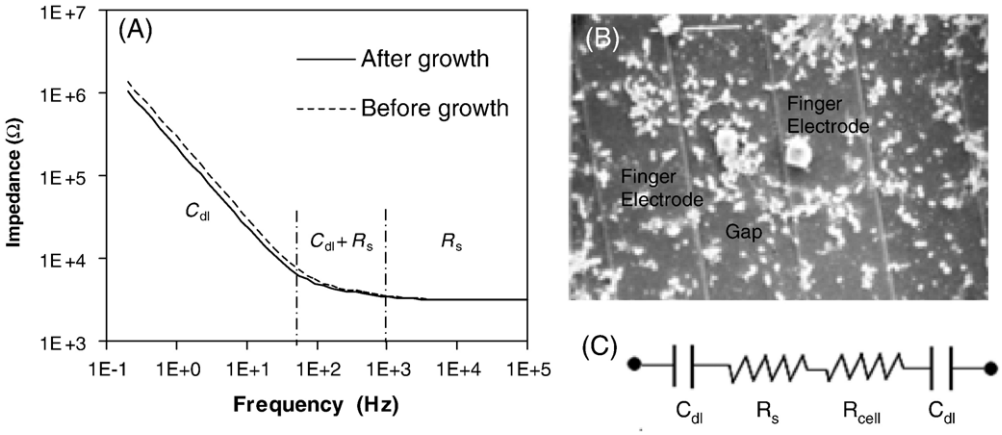

Fig. 3. (A) Impedance spectra of the IDA sensor recorded before and after *S. typhimurium* growth in SC/M/T medium in the frequency range from 0.2 Hz to 100 kHz. Initial cell number: 1.76×10^2 CFU/ml. (B) SEM image of bacteria attachment on the IDA electrode (Reprinted with permission from Biosensors and Bioelectronics and kind permission from Yang et al., 2004a). (C) Modified equivalent circuit of IDA after bacteria attachment.

with the plate count method were obtained compared with conductance methods. However, the interface capacitance measured at 1 kHz also contained contributions due to the geometry (roughness) of the electrodes and the electrochemical characteristics of the interface.

2.5. Interdigitated array microelectrodes (IDAs) in impedance measurements

Generally, microelectrodes have great advantages over conventional electrodes for analytical measurements, such as low resistance, high signal-to-noise ratio, rapid attainment of steady state, and the use of small solution volumes (Stulik et al., 2000). In recent years, microfabricated interdigitated array (IDA) microelectrodes have received great attention in the areas of impedimetric immunosensing and biosensing (Van Gerwen et al., 1998; Laureyn et al., 1999a,b, 2000), and impedance measurement for studies of biological cell behaviors (Ehret et al., 1997, 1998).

An IDA consists of a pair of microband array electrodes that mesh with each other. These two sets of microelectrodes can be the two poles in a bipolar impedance measurement setup. More attractively, there are multiple electrode pairs in an IDA, and the distance between finger electrodes can be in micron to nanometer range in order to probe the volume close to the electrodes.

Recent studies have demonstrated the promise of interdigitated microelectrodes in impedance measurements for monitoring the growth of bacteria (Yang et al., 2004a; Yang and Li, 2006). Regarding the equivalent circuit, the circuit in Fig. 2B still applies to the IDA impedance measurement systems. However, the frequency range for each region may vary because of the difference in size and dimension of the electrodes. Yang et al. (2004a) showed that the double layer region appeared in a lower frequency range (<100 Hz) in the IDA system (Fig. 3A) when compared with a conventional electrode system (<10 kHz) (Fig. 2B), and the solution resistance region was in the frequency range from 100 Hz to 10 kHz. By using the IDA, the difference in impedance before and after bacterial growth was observed at frequencies below 100 Hz, but was negligible at frequencies higher than 1 kHz (Fig. 3A). By simulating the measured data to the equivalent circuit, changes in double layer capacitance due to bacterial growth was about 30%, while changes in solution resistance was negligible (−0.58%) (Yang et al., 2004a). In this respect, the IDA system is quite different from the conventional electrode systems in that the conventional electrode systems generally measure the change in solution resistance, while IDA electrode systems actually measure the double layer capacitance to monitor bacterial growth. The possible reason accounting for this difference is the attachment of bacteria cells on the IDA surface. In an IDA system, bacteria cells in the growth medium have more opportunities to become attached to the IDA electrode surface since the IDA has a relatively large surface area and the system has a large surface to volume ratio. Yang et al. (2004a) observed a high coverage of attached *Salmonella* cells on an IDA electrode after the electrode was used in impedance detection of *Salmonella* growth (Fig. 3B). However, bacteria cells attached to the electrode surface were usually separated by a gap of 10–20 nm between the cell membrane and the electrode surface (Gingell, 1990). The aqueous gap prevented a direct influence of the cell membrane capacitance on the electrode impedance. Cell membrane resistance of these attached bacterial cells acted as resistors connected in series with medium resistance in the equivalent circuit to compensate for the decrease in medium resistance. Taking these attached cells into consideration, the equivalent circuit for an IDA system after bacterial growth can be modified as shown in Fig. 3C.

The advantages of the IDA impedance system include a reduction of the sample volume and a more rapid detection time. With the IDA system, the test volume can be reduced from 10–15 ml down to 1–2 ml. Based on our studies, it also reduces the assay time effectively as shown in Fig. 4. The detection time for the same initial concentration of bacteria is reduced by 3–4 h by using the IDA system when compared with the conventional electrode system.

Varshney and Li (in press) reported a double interdigitated array microelectrode-based impedance sensor for detection of viable *E. coli* O157:H7 in a growth medium. They used a detection flow cell which had two set of IDA electrodes, one on

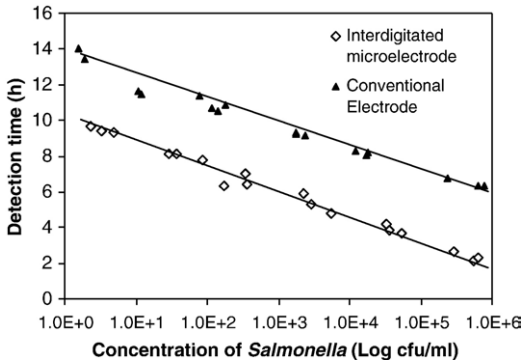

Fig. 4. Comparison of detection times obtained by the interdigitated microelectrode system and the conventional electrode system in impedance measurement for bacterial growth. (Derived from Yang et al., 2004a and Yang et al., 2003).

the top and one on the bottom surfaces of the flow cell, to enhance the sensitivity of the impedance measurement. *E. coli* O157:H7 cells were grown in a low conductivity yeast-peptone-lactose-TMAO medium outside the flow cell. After bacterial growth, impedance of the cell culture was measured inside the flow cell. This sensor successfully detected *E. coli* O157:H7 in a range from 8.0 to 8.2×10^8 cfu/ml after an enrichment growth of 14.7 h and 0.8 h, respectively. It was expected that the double IDA sensor could improve the sensitivity of impedance detection by providing more surface area as compared to a single a single IDA sensor (Varshney and Li, 2007, in press).

2.6. Microchips for impedance detection of bacteria

More recently, miniaturization of impedance detection system into a chip-based device has shown great promising in rapid detection of bacterial growth. Gomez and coworkers (Gomez et al., 2001, 2002, 2005) were among the first to fabricate integrated silicon-based biochips for impedance detection of microbial metabolism. The basic idea was to confine a few live bacterial cells into a small volume on the order of nano- to pico-liters, so that the metabolism of a few live cells in a low conductivity buffer can be rapidly detected by impedance measurement using interdigitated microelectrodes. The threshold of detection can be reduced, thus allowing detection of metabolism of a few cells.

However, confinement or capture of cells into a small volume is a challenge. A new technique of "Impedance microbiology-on-a-chip" has been demonstrated by Gomez and coworkers (Gomez et al., 2005). This on-chip impedance microbiology method innovatively integrated a technique called dielectrophoresis (DEP) into the chip to concentrate bacterial cells from diluted samples into a very small volume. DEP is the electrokinetic motion of dielectrically polarized particles in non-uniform electric fields (Pohl, 1978). As most biological cells behave as dielectric particles in an external electric field, DEP allows the manipulation of the biological cells in a liquid suspension. Particularly, recent progress in the development of microelectrode structures has made DEP a very useful technique for manipulation of biological cells in microfluidic devices, biochips and biosensors; some applications are illustrated in Section 3.4 in this review. The impedance microbiology-on-a-chip developed by Gomez et al. (2005) contained two sets of interdigitated microelectrodes (Fig. 5A). One set was for dielectrophoretically capturing bacterial cells from the flow into the small chamber, and the other set was for monitoring the impedance change when bacterial cells grew in the chamber. The design concept was to use DEP to deviate the bacterial cells from a main channel into a small channel that led the cells into a measurement chamber which had a volume of 400 pl. Their metabolism was measured by the set of impedance measurement interdigitated microelectrodes. Gomez et al. (2005) showed a representative image of fluorescence labeled *Listeria* cells concentrated by DEP into the picoliter measurement chamber (Fig. 5B). The concentration factor of this chip was between 10^4 and 10^5 when the cells in an original sample volume of 40 µl was concentrated into the 400 pl chamber, provided that 10–100% of the cells were captured by DEP. The significant improvement in detection time was demonstrated by the comparison of the impedance growth curves of *Listeria* cells in Luria–Bertani (LB) medium in the chip with and without DEP concentration

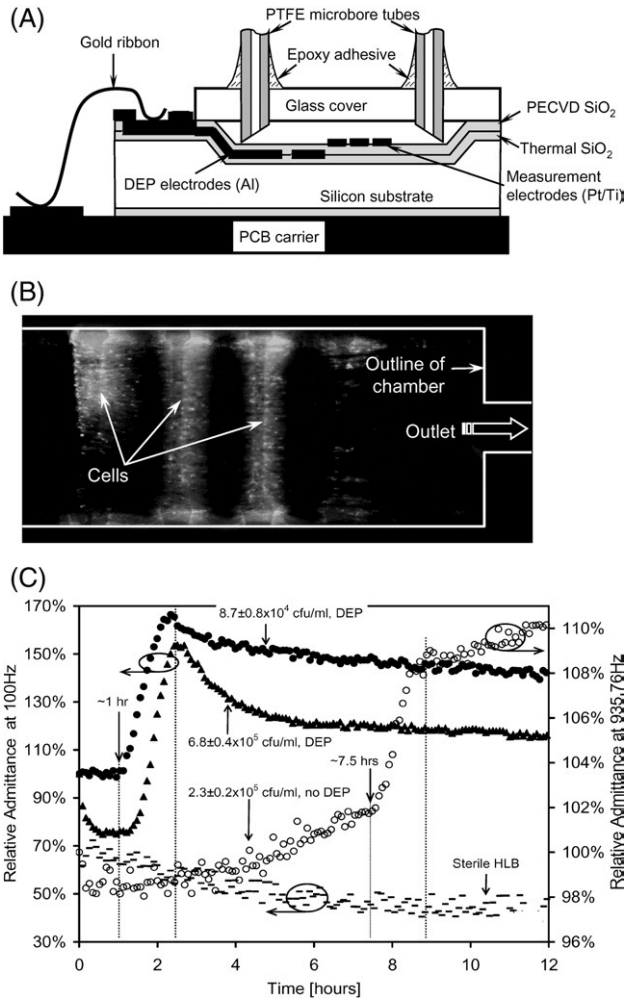

Fig. 5. (A) Cross section of the biochip for trapping cells and monitoring bacterial growth. (B) Fluorescently labeled *Listeria* cells trapped in the nano-chamber by DEP. (C) Impedance growth curves of *Listeria* cells growing LB medium in the biochip (reprint from Gomez et al., 2005).

(Fig. 5C). The bacterial sample containing ~ 6.8×10^5 cfu/ml with the DEP concentration presented an impedance metabolic signal indicating the exponential growth at approximately 1 h, while the sample containing similar concentration of cells without DEP concentration needed approximately 7.5 h to produce a detectable impedance signal. Such concentration technique in micro-fabricated chips eliminates the need to enrich the bacterial population by long culture steps in conventional cell culture methods, and can drastically reduce the total assay time. This on-chip impedance microbiology has achieved the detection time of 1 h for a sample with a starting concentration of 10^4 cfu/ml (Gomez et al., 2005).

Spiller et al. (2006) developed a microsystem for growth inhibition test of *Enterococcus faeccalis* based on impedance measurement. They designed a device containing a 2×5-array of conductivity measurement chambers using the 1536 microtiter plate format. With this miniaturized system, a reduction of sample volume from 50 ml down to 12 μl was achieved. Each chamber was equipped with a sensor system including three electrodes in a ring structure which were covered by a membrane layer, not allowing the bacterial cells to penetrate the membrane. By applying the current in the middle of the three electrodes, the response current through the electrolyte and the suspension can be measured by the two measurement electrodes. Cell growth curves of *E. faecalis* with different concentrations of amoxicillin were monitored by this sensor system, showing the growth inhibition of the bacterial cells by antibiotic amoxicillin at 8 mg/l. The miniaturized biosensor gets the results in less than 2 h, in comparison to the standard test that can get the result after 6 h.

Sengupta et al. (2006) developed a micro-scale multi-frequency reactance measurement technique to detect bacterial growth. They designed a micro-capillary to increase the bulk resistance (R) of the medium, thus increasing its RC time constant. This method permitted the detection of an initial concentration of ~ 100 cfu/ml of *E. coli* within 3 h of incubation.

3. Impedance biosensors for bacterial detection

A novel impedance biosensor for bacterial cell detection is constructed by immobilizing antibodies that are specific to the target bacterial cells on an electrode surface. The sensor probes the attachment of bacterial cells by measuring the change in electrical properties of the sensor due to the insulating properties of the cell membrane. The presence of intact cell membranes on the electrodes determines the current and thus the sensor signal. The impedance of the sensor is generally measured as a function of the interrogating frequency, namely electrical/electrochemical impedance spectroscopy (EIS). The measurement of the impedance can be performed in the presence or absence of a redox probe, which are referred to as Faradaic and non-Faradaic impedance measurements (Bard and Faulkner, 1980); wherein one or other type dominates the total impedance signal. Without a redox probe, the measured impedance signal resulted directly from the intact bacteria cells that are adherently growing on or physically attached to the electrode surface, mainly owing to the insulating effects of the cell membranes (Ehret et al., 1997, 1998). The impedance is influenced by the changes in number, growth and morphological behavior of adherent cells. In the presence of a redox probe, the sensor probes the biological events occurring on its surface by measuring the induced changes in Faradaic impedance. This technique has been regarded as an effective way to sense the formation of antigen–antibody (Kharitonov et al., 2000; Maupas et al., 1996; Patolsky et al., 1998; DeSilva et al., 1995), biotin–avidin complexes (Athey et al., 1995), oligonucleotide–DNA interaction (Bardea et al., 1999), and the binding of foot-and-mouth disease virus (Rickert et al., 1996) on electrode surfaces by probing the features of the interfacial properties (capacitance, electron transfer resistance) of electrodes.

3.1. Impedance properties of biological cells

Biological cells consist of a complex spatial arrangement of materials that have very different electrical properties. The cell membrane consists of a lipid bilayer containing many proteins, where the lipid molecules are oriented with their polar groups facing outwards into the aqueous environment, and their hydrophobic hydrocarbon chains pointing inwards to form the membrane interior. The inside of a cell contains membrane-covered particulates, such as mitochondria, vacuoles and a nucleus, and many dissolved charged molecules. While the cell membrane is highly insulating, the interior of the cell is highly conductive. The conductivity of the cell membrane is around 10^{-7} S/m, whereas the conductivity of the interior of a cell can be as high as 1 S/m (Pethig and Markx, 1997). Considering each single ion channel as a resistor and the total membrane resistance as the parallel combination of all the ionic channels, the overall membrane resistance can range from 1 M Ω to 100 G Ω μm^2 depending on the cell type and the location of the patch of the membrane (Borkholder, 1998), based on the individual channel resistances and density ranges for various tissue and channel types found in literature (Hille, 1992). The membrane capacitance is approximately 0.01 pF/μm^2 considering the thickness of most biological cell membranes as 8 nm (Hille, 1992). Pethig (1979) also reported that natural cell membranes (thickness 5–10 nm) show a membrane capacitance of 0.5–1.3 μF/cm^2 and a membrane resistance of 10^2–10^5 Ω·cm^2. These data are in agreement with each other.

If cells attach on an electrode surface, they would effectively reduce the electrode area that the current reaches and hence increase the interface impedance. Ehret et al. (1997, 1998) demonstrated the insulating property of the cell membrane with a sensor based on impedance measurement of adherently growing cells on interdigitated microelectrodes. The study showed that the presence of intact cell membranes on the electrodes determined the current flow and thus the sensor signal. The density, growth and long-term behavior of cells on the electrodes changed the impedance of the sensor. Luong et al. (2001) developed an impedance sensor for monitoring motility, spreading, and mortality of adherent insect cells. Upon the attachment and the spreading of cells on the gold electrode, the

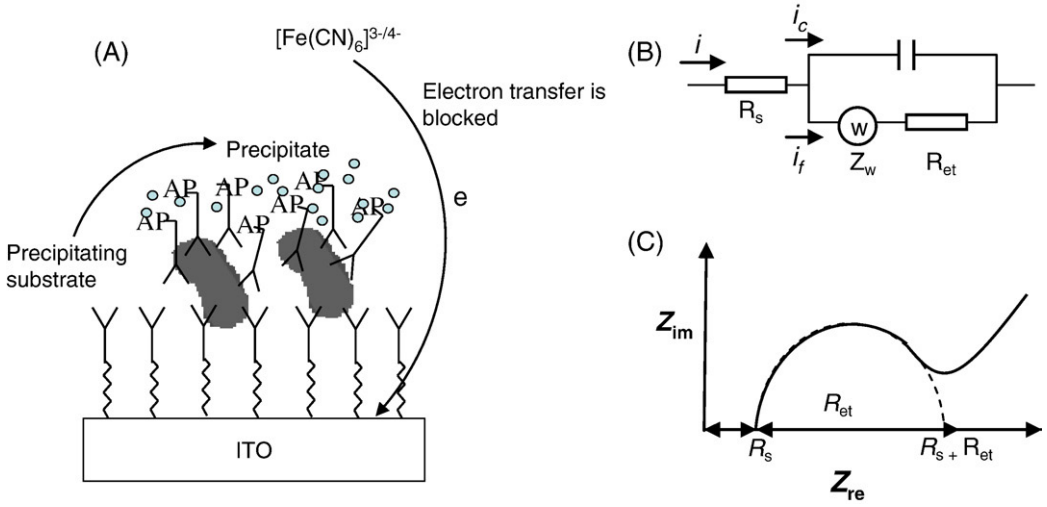

Fig. 6. (A) The principle of the electrochemical impedance biosensor for bacteria detection with enzymatic amplification. (B) The Randles model equivalent circuit for the electrochemical impedance biosensor, and (C) The typical Nyquist plot (Z_{im} vs. Z_{re}) of Faradaic impedance spectrum of electrochemical cell in presence of redox probe. (Modified from Ruan et al., 2002).

impedance increased because the cells acted as insulting particles to restrict the current flow.

3.2. Electrochemical impedance biosensors using redox probes

The electrochemical impedance spectroscopic biosensor for bacterial detection was first reported in 2002 (Ruan et al., 2002). The principle of the biosensor was based on the measurement of Faradaic impedance in the presence of a redox probe, $[Fe(CN)_6]^{3-/4-}$, upon the attachment of the bacterial cells to the electrode surface (Fig. 6A). Anti-*E. coli* antibodies were immobilized on a planar indium-tin oxide (ITO) electrode to capture *E. coli* cells to the electrode surface. This biosensor also involved an amplification step in which secondary antibodies with horseradish peroxide was applied to produce precipitation of insoluble products on the electrode surface. Such a precipitate layer would effectively block the electron transfer and thus amplified the signal (Fig. 6A).

The behavior of the impedance biosensor system can be well interpreted by the Randles model equivalent circuit shown in Fig. 6B. The equivalent circuit consists of ohmic resistance (R_s) of the electrolyte, double layer capacitance (C_{dl}), electron-transfer resistance (R_{et}) and Warburg impedance (Z_w) of the electrode. The parallel elements are introduced because the total current through the working electrode is the sum of distinct contributions from the Faradaic process (i_f) and double layer charging (i_c). Since all the current must pass through the uncompensated solution resistance, R_s is inserted as a series element in the equivalent circuit. Among these electrical elements in the equivalent circuit, R_s and Z_w represent bulk properties of the electrolyte solution and diffusion of the redox probe, whereas C_{dl} and R_{et} depend on the dielectric and insulting features at the electrode/electrolyte interface, and they are affected by the property change occurring at the electrode interface.

R_{et}, the electron transfer resistance, is the parameter that is measured in the biosensor. The attachment of bacterial cells would retard the interfacial electron-transfer kinetics and increase the electron-transfer resistance. The total electron-transfer resistance after cell attachment can be expressed as

$$R_{et} = R_e + R_{cell} \quad (4)$$

where R_e and R_{cell} are the electron-transfer resistance of the antibody-immobilized electrode and the variable electron-transfer resistance introduced by the attached bacterial cells.

Among many of the impedance plots, Nyquist plots (Z_{im} vs. Z_{re}) is the best way to visualize and determine the electron transfer resistance, R_{et}. A typical Nyquist plots (Fig. 6C) is comprised of a semicircle lying on the Z_{re} axis continued with a straight line (Bard and Faulkner, 1980). The semicircle portion observed at the higher frequencies corresponds to the electron-transfer-limited process, while the linear part is characteristic of the lower frequencies and represents the diffusion-limited processes. Thus, the electron-transfer kinetic parameters and diffusion characteristic can be extracted from the semicircle and linear parts of the impedance spectra, respectively. The intercept of the semicircle with the Z_{re} axis at high frequency is equal to R_s. Extrapolation of the semi-circle to lower frequency yields another intercept with the Z_{re} axis equaling $R_s + R_{et}$. The diameter of the semicircle is equal to the electron-transfer resistance, R_{et}.

Ruan et al. (2002) showed that the electron transfer (R_{et}) increased with the increasing cell concentration. The biosensor had a linear response range in the bacterial concentration from 6.0×10^4 to 6.0×10^7 cells/ml with a detection limit of 6.0×10^2 cells/ml.

Label-free electrochemical impedance biosensors can be achieved by using the interdigitated array microelectrode (IDA). Several studies have shown that IDA microelectrodes have great promises in the field of label-free impedimetric biosensing (Laureyn et al., 1999a,b; Van Gerwen et al., 1998). The IDA microelectrodes are capable of monitoring the changes of the electrical properties in the immediate neighborhood of their

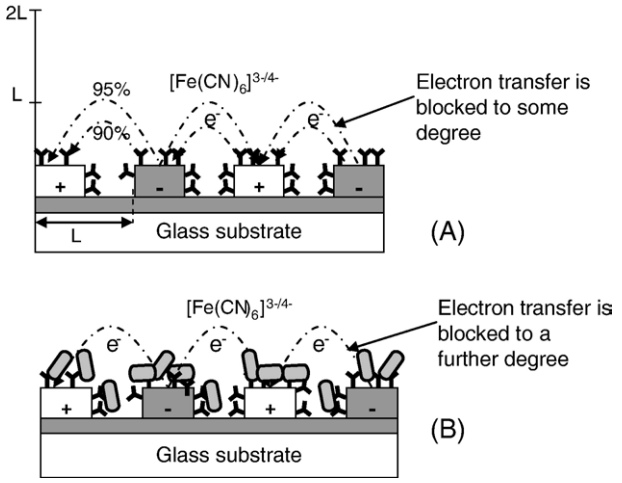

Fig. 7. (A) Schematic of current comprisement of interdigitated electrodes-based biosensor. *L* is the electrode width plus the spacing. A curve of e.g. 95% means that 95% of the current flows beneath the curve. (B) Principle of the direct impedance immunosensor, electron transfer is blocked by bacterial cells bound to the sensor surface. (⬭) stands for *E. coli* O157:H7 cell, (Y) stands for anti-*E. coli* antibody. (Modified from Yang et al., 2004b).

surfaces. It is reported that 95% of the current between the two finger electrodes in an IDA flows above the electrode surface within a distance that equals the sum of electrode width and space (Fig. 7A) (Van Gerwen et al., 1998). Therefore, the size of the IDA electrode must be chosen for different biological entities in consideration of their sizes (could be nanometer to micrometer) to achieve a sensitive biosensor for that type of biological entity. For example, micrometer IDA electrodes are suitable for sensing of biological cells, while nanometer IDAs are more proper for sensing DNA.

Yang et al. (2004b) reported a label-free electrochemical impedance immunosensors for detection of *E. coli* O157:H7 using interdigitated microelectrodes. The IDA consisted of a pair of microband array indium-tin oxide (ITO) electrodes. Each electrode had 25 digital pairs with 15 μm digit width, 15 μm interdigit space and a digit length of 2,985 μm. Anti-*E. coli* antibodies were immobilized onto an indium-tin oxide (ITO) interdigitated microelectrode through covalent linkage between carboxyl groups on the antibodies and the abundant reactive hydroxyl group on the ITO surface. During the Faradaic process of the oxidation and reduction of the redox probe ($[Fe(CN)_6]^{3-/4-}$), electrons were transferred between the two sets of the microarray electrodes through the redox probe. When bacterial cells were attached to the electrode surface, they inhibited the electron transfer between the electrodes, thus an increase in the electron transfer resistance was expected (Fig. 7B). Compared to the size of a bacterial cell, 1–2 μm long with ~ 0.5 μm in diameter, the dimensional compatibility of the IDA made it an obvious choice for its ability to sensitively detect the immobilization of antibody and the capture of bacterial cells on the electrode surface without any amplification step. This label-free biosensor achieved a response range to *E. coli* concentrations from 10^5 to 10^8 cfu/ml, with a detection limit of 10^6 cfu/ml. Although the detection of this label-free biosensor was not as low as that of the impedance biosensor with enzyme amplification (Ruan et al., 2002), its detection limit was comparable with other label-free immunosensors for detection of pathogenic bacteria using different transducer techniques, including QCM (quartz crystal microbalance) immunosensors for detection of *Salmonella* with detection limits of 3.2×10^6 cfu/ml and 9.9×10^5 cfu/ml (Park et al., 2000; Park and Kim, 1998), SPR (surface plasmon resonance) immonusensors for detection of *Salmonella enteritidis* and *L. monocytogens* with detection limits of 10^6 cfu/ml (Koubova et al., 2001), and a SPR sensor for detection of *E. coli* O157:H7 with a detection limit of 10^7 cfu/ml (Fratamico et al., 1998). Apparently, the response range and the detection limit of the biosensor could be optimized by the size, dimension, and design of the IDA.

3.3. Non-Faradaic impedance biosensors for bacterial detection without redox probes

Non-Faradaic impedance biosensors perform impedance measurement in the absence of any redox probe. Bacteria detection is based on the impedance change upon the attachment of bacterial cells on an interdigitated microelectrode in the absence of any redox probe in the sample solution. Radke and Alocilja (2005) reported an impedance biosensor for the detection of *E. coli* O157:H7 using a high density microelectrode array without using any redox probe in the detection system. The sensor contained a total of 1700 finger electrodes with an electrode width of 3 μm and 4 μm spacing between each electrode to provide a large active area. To immobilize antibody, the gold electrode surface was silanized with 3-mercaptomethyldimethylethoxysilane (MDS) in dry toluene, and followed by functionalization of the crosslinker, *N-y*-maleimidobutyryloxy succinimide ester (GMBS). Polyclonal antibodies specific to *E. coli* O157:H7 at a concentration of 150 μg/ml were then immobilized to the sensor surface through the crosslinker. When the biosensor was immersed into the bacteria suspension in 0.1% peptone water, bacterial cells were attached to the immobilized antibodies on the sensor surface. The increase in impedance caused by the bound bacteria on the electrode surface due to the insulating property of the cell membrane was monitored over a frequency range of 100 Hz–10 MHz. The sensor was able to discriminate between cell concentrations of 10^4–10^7 cfu/ml.

Other researchers have continuously improved the impedance biosensor methods by integrating newly developed nanoparticles and microfuidics with interdigitated microelectrodes. Varshney and Li (2007) reported an IDA-based impedance biosensor coupled with magnetic nanoparticle–antibody conjugates for rapid and specific detection of *E. coli* O157:H7 in ground beef samples. Instead of immobilizing antibodies directly on the electrode surface, antibodies were immobilized on magnetic nanoparticles. Magnetic nanoparticles (Fe_3O_4, diameter ~ 145 nm) were conjugated with anti-*E. coli* antibody through biotin–streptavidin chemistry. The conjugates were then used to separate and concentrate *E. coli* cells from ground beef samples. The nanoparticle–cell complexes in 0.1 mannitol solution were measured by impedance using IDA microelectrodes with 50 pairs of

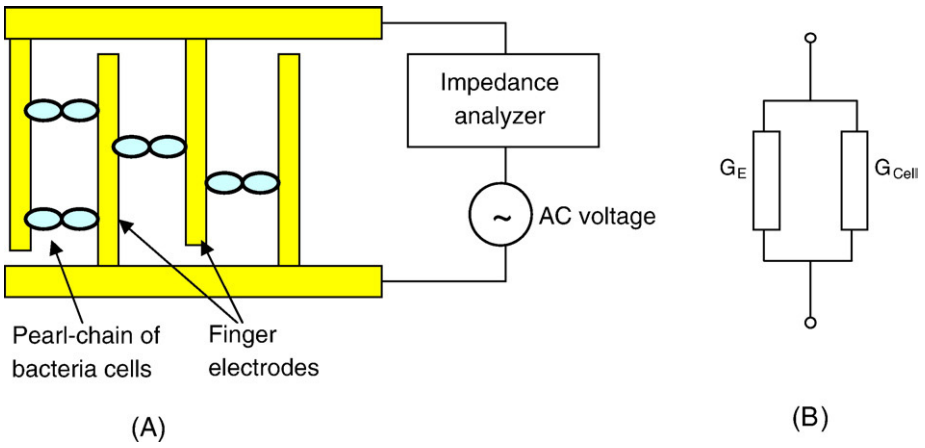

Fig. 8. (A) A schematic diagram of impedance detection of bacterial cells formed in a chain on an interdigitated microelectrode and (B) the equivalent circuit of the pearl-chains of bacteria trapped between the finger electrodes, only showing the conductance component of the admittance for simplicity. G_E: conductance of the intact electrode, and G_{cell}: the conductance of the trapped bacterial chains. (B) is reprinted from permission of Sens. Actuators B and kind permission from Suehiro et al., 2003b).

fingers electrodes each measuring 15 μm in width and space. When 2 μl of the complexes solution was spreading on the IDA electrodes surface, nanoparticle–cell complexes were concentrated into the active layer of the IDA with the assistance of a magnet field. The lowest detection limits of this biosensor system for detection of *E. coli* O157:H7 in pure culture and ground beef were 7.4×10^4 and 8.0×10^5 cfu/ml. This biosensor method was late refined into a microfluidic chip-based biosensor by the same group (Varshney et al., 2007). The microfluidic chip had a small detection chamber (60 nl) formed by a poly (dimethylsiloxane) (PDMS) with embedded gold interdigitated microelectrodes on the bottom of the chamber. Magnetic particle–cell complexes in mannitol solution were injected into the detection chamber for sensitive impedance measurement. This microfluidic impedance biosensor was able to detect as low as 1.6×10^2 and 1.2×10^3 cfu/ml of *E. coli* cells present in pure culture and ground beef samples, respectively. Boehm et al. (2007) reported an on-chip microfluidic biosensor for *E. coli* detection and identification. In this microfluidic biosensor, anti-*E. coli* antibodies were immobilized on the glass surface which served as the bottom of the microfluidic chamber, the impedance detection electrodes were however on the top cover of the chamber. Bacteria in suspension passing through the microfluidic chamber were selectively recognized and captured by the immobilized antibodies, thereby increasing the measured impedance within the chamber. This biosensor was able to detect ~ 10^4 cfu/ml of *E. coli* when a shallow chamber (2 μm) was used.

These studies have significantly advanced the state of the art in impedance biosensors for the detection of pathogenic bacteria and have fostered the integration of different techniques with biosensors. First, these studies have indicated that antibodies for specific recognition of target cells are not necessary immobilized directly on the sensor (e.g. electrode) surfaces. Instead, microbeads or nanoparticles coated with desired antibodies can serve as the recognition element in the biosensor systems. The use of microbeads or nanoparticles can improve the capture efficiency of antibodies to target cells (Varshney et al., 2007). Second, the microfluidic-based sensors allow continuous injection/perfusion of bacteria samples and accumulation/concentration of bacterial cells inside the impedance detection chamber over time, which can enhance the detection sensitivity and is particular useful for detecting low concentrations of bacteria (Boehm et al., 2007). Third, these studies have brought attention to the chamber height or the "active layer" of the microelectrodes in impedance detection. The microelectrodes scan a region that is only a few microns above its surface, which is referred to as the "active layer" in which impedance can be sensitively detected. Therefore, a thin microfluidic chamber right above the electrode surface reduces the time required for conductive ions to diffuse to reach the "active layer", thus resulting in a more rapid kinetic reaction (Varshney et al., 2007). The use of magnet field to confine nanoparticle–cell complexes on the surface of IDA and the use of a shallow chamber in these above mentioned microfluidic biosensors have demonstrated the enhanced sensitivity of impedance detection by bringing detection targets into the "active layer" or reducing the chamber height. Of course it should be noted that reducing the chamber height will also result in a longer time to flow the sample in a microfluidic configuration.

3.4. Impedance coupled with dielectrophoresis and electropermeabilization

Another impedance measurement to detect the presence of bacterial cells on an interdigitated microelectrode is dependent on the difference of conductance or admittance (reciprocal of impedance) between the cells and the medium (Suehiro et al., 2003a, 2003b). A schematic diagram of impedance detection of bacterial cells and the equivalent circuit of the pearl-chains of bacteria trapped between the finger electrodes are shown in Fig. 8 (Suehiro et al., 2003a). Only the conductance component of the admittance is shown for simplicity. The bacterial cells are trapped between the finger electrodes and form a cell chain to link the finger electrodes. The apparent electrode conductance,

G_T is the sum of the conductance of the intact electrode, G_E and the conductance of the trapped bacterial chains, $G_{cell,}$ given by Eq. (5).

$$G_T = G_E + G_{cell} \quad (5)$$

It is assumed that the electrode conductance G_E does not change over time. The conductance of the cells, G_{cell}, is related to the number of cells which form the pearl-chain, m, the conductance of the bacterial cell, G_B, and the total number of cells trapped by the electrode, N.

$$G_{cell} = N^* G_B / m \quad (6)$$

If the trapped cells possess higher admittance than the suspension medium, the total admittance of the microelectrode increases with the increasing of the number of trapped cells. Cell concentration in a suspension can be quantitatively estimated based on the analysis of increment rate of admittance.

The formation of the bacterial chains between the finger electrodes can be achieved by using dielectrophoresis (DEP) technique. The advantages of DEP concentration and antibody selective capture have been combined to demonstrate selective capture of target cells from a mixture of cells with similar dielectric properties in a microfluidic biochip (Yang et al., 2006). The device consisted of an array of interdigitated electrodes on a flat silicon substrate and a 16 μm high micro-channel within a PDMS cover. *L. monocytogenes* V7 cells were used to demonstrate the function of the microfluidic biochip. Positive DEP (at $20V_{pp}$ and 1 MHz) was used to concentrate bacterial cells from the fluid flow. Without DEP, no *L. monocytogenes* cells could be seen on the channel surface. DEP collected ~ 90% of the cells in a continuous flow at a flow of 0.2 μl/min into the micro-channel with concentration factors between 10^2 and 10^3, in sample volumes of 5–20 μl. High flow rate at 0.6 μl/min reduced the DEP capture efficiency to ~ 65%. Higher capture efficiencies of 99% or greater could be achieved by optimizing the channel dimension along with the flow rate. Selective capture of *L. monocytogenes* from the samples was achieved by immobilizing monoclonal anti-*Listeria* antibody onto the surface of DEP chamber through biotin–streptavidin chemistry. The antibody capture efficiency was between 20% and 30% for cell numbers in the range on 10^1 to 10^4 cfu/5 μl. With this device, the lowest number of cells captured by DEP from a 5 μl of the sample in this study was about 60 cells, when DEP was turned off, about 16 cells were captured by the antibodies on the channel surface. This result indicates that DEP is capable of capturing very few bacterial cells in a microfluidic device.

By combining the DEP technique and impedance measurements, Milner et al. (1998) and Suehiro et al. (1999) reported a detection technique called dielectrophoretic impedance measurement (DEPIM) which utilizes the positive dielectrophoretic force to trap suspended biological cells onto an interdigitated microelectrode array to form pearl-chains. These pearl-chains are electrically connected in parallel to the electrode gap and therefore increase the conductance and the capacitance between the finger electrodes. Cell population can be quantitatively evaluated by monitoring the change in the electrode impedance or admittance.

For selective detection of bacterial cells, Suehiro et al. (2003a) combined this DEPIM method with antigen–antibody reaction to realize selective detection of *E. coli*. There were two ways to utilize the antigen–antibody reaction in this detection system. In one way, antibody can be added to the cell suspension after dielectrophoretic trapping of bacteria to cause agglutination of target bacteria. Agglutinated bacteria, whose apparent size increase, experienced greater DEP forces which trap them in the gap of the electrodes, while other non-agglutinated non-target bacterial cells were washed out in the wash steps. In the other way, anti-*E. coli* antibodies can be immobilized onto the electrode surface so that only antibody-specific bacteria would be bound to the immobilized antibodies (Suehiro et al., 2006). Both ways allowed target bacterial cells to be selectively trapped between the electrode gap so that the change in impedance signal would correspond only to the target bacteria.

The same group (Suehiro et al., 2005) reported an improved DEPIM method for detection of *E. coli* by combining this technique with electropermeabilization. *E. coli* cells in suspension were captured by positive dielectrophoretic force onto an interdigitated microelectrode array. After *E. coli* cells were trapped by DEP, electropermeabilization (EP) was performed by applying a high AC electrical field to the trapped bacteria which led to intracellular ion release through damaged cell membranes, and a resulting conductance increase. By this method, 10^2 cfu/ml of *E. coli* was detected in 3 h.

4. Conclusions and perspectives

Impedance technique as a principle of transduction has become a fertile area for developing rapid and effective methods for the detection of bacteria. Although classic impedance microbiology was established a long time ago and has been developed as a rapid automated method for bacteria detection in 24 h, impedance technique is now entering into a new stage as a chip-based method. Advances in microfabrication have paved the way for miniaturization of impedance microbiology into microdevices and biochips, which have been proved to be very successful in maximizing the impedance signal, minimizing the volume of testing sample, increasing sensitivity, and saving assay time for the detection of foodborne pathogenic bacteria. Attractively, impedance technique is one of the few techniques that allows growth-based detection of bacteria, which can differentiate dead cell vs. live cells. Differentiation and detection of live cells is more useful than detection of both live and dead cells, since dead cells are usually not pathogenic. The ability to differentiate live and dead cells has the great promises to significantly improve food safety and security and to benefit health care.

To date, impedance biosensors for bacterial detection are comparable with current well studied rapid methods such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction which have achieved detection limits varying from 10^1 to 10^6 cfu/ml and other various biosensors that have achieved detection limit of 10^3 to 10^4 cfu/ml with the assay time around 2 h under ideal conditions.

Impedance techniques have the great potential to solve the two key issues/concerns in detection of foodborne pathogens: rapidity and detection limit. Rapidity includes assay time and number of samples per operation (Fung, 1992). While microchip-based impedance microbiology can effectively reduce the assay time, high throughput microchips would greatly increase the number of samples per operation. It is possible to design the impedance measurements in a 96 to 1536 microplate format, which would allow continuous monitoring and high throughput screening. Miniaturized devices reduce the handling effort and cost, and provide a faster, space-saving and more economical accomplishment.

Single cell level detection need to be explored using impedance biosensors. Despite the significant improvement in reducing the assay time and lowering detection limit, to date, detection at a single cell level within a short assay time has not been achieved yet. As demonstrated in micromachined impedance spectroscopy flow cytometer (Gawad et al., 2001), a microdevice can be designed to measure the spectral impedance of individual cells, which have shown the possibility for individual cell detection in a high throughput way by impedance technique.

Impedance biosensors also offer the promises in developing biosensor array for multiplex analysis of different bacteria simultaneously, providing label-free, on-line and high throughout devices for bacteria detection.

By successfully integrating micro and nano-fluidics with biosensors, many of the unit operations associated with sample preparation, such as separation (cell or biomolecule), mixing, incubation, concentration, etc., may be performed directly in a lab-on-a-chip format. Also, the use of microliter and nanoliter volumes implies the use of smaller sample volumes, smaller quantities of usually costly reagents, and greater analytical sensitivity.

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