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Electrochemical biosensors for rapid detection of *Escherichia coli* O157:H7

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ABSTRACT

Electrochemical biosensors have shown great promise in the development of rapid methods for the detection of foodborne pathogens and have been intensively studied over the past two decades. The scope of this review is to summarize the advancements made in the development of electrochemical biosensors for the rapid detection of one of the most common foodborne pathogens, *Escherichia coli* O157:H7. The article is intended to include different configurations of electrochemical biosensors based on the sensing principles and measured electrical parameters, as well as the latest improvements of technology in the progress of electrochemical biosensor development to detect *E. coli* O157:H7. By discussing the current and future trend based on some of excellent published literatures and reviews, this survey is hoped to illustrate a broad and comprehensive understanding of electrochemical biosensors for the detection of foodborne pathogens.

Keywords: Electrochemical biosensors, Rapid detection, Detection strategy, *Escherichia coli* O157:H7.

1. INTRODUCTION

Foodborne illnesses caused by pathogenic bacteria have always been a serious threat to the health of people and to the economy of nations. The Centers for Disease Control and Prevention (CDC) has estimated that roughly 48 million people get sick, 128,000 are hospitalized, and 3,000 are dead every year in the United States due to foodborne illness [1]. Shiga toxin-producing *E. coli* (STEC) serotype O157:H7 are the most commonly mentioned in *E. coli* group in association with foodborne outbreaks. In the United States, there are estimated over 63,000 cases of *E. coli* O157:H7 infection occurred each year, about 2,100 of those cases involve hospitalization and up to 20 deaths occur. Especially due to the increasing consumption of minimally processed products, such as fruits, vegetables, and ready-to-eat (RTE) products, multistate foodborne outbreaks related to this pathogen in recent years have caused

serious concerns on the inspection and monitoring to ensure the safety of food products and reduce the occurrence of foodborne illness [2].

Even though there have been many legislations, regulations, and methodical programs like good manufacturing practices (GMP) and hazard analysis and critical control point (HACCP) which can greatly reduce the occurrence of *E. coli* O157:H7 in food, the methods for sensitive, reliable, and rapid detection of this pathogen are still critical to identify and prevent the problems. Currently, culture and molecule based conventional methods for the detection and enumeration of *E. coli* O157:H7 are still the most commonly practiced techniques in the food safety area. Briefly, the culture-based methods rely on the multiplication of all viable bacterial cells on a nutrient rich medium that supports the growth of the organisms to form colonies that can be enumerated. In recent years, the culture-based methods have been modified to suit for the different applications with the development of automatic enumeration system [3-5]. On the other hand, molecule especially polymerase chain reaction (PCR) based methods show high level of specificity and sensitivity because this type of methods is based on the detection of nucleic acid sequences of target bacteria, and the specifically designed short synthetic oligonucleotides can solely hybridize to these target sequences that are complementary to each other [6]. Furthermore, the amplification time of the DNA from the bacterial cells can be much shorter, which leads to a much more rapid procedure to detect the bacteria than conventional methods [4, 7-9]. Some of the protocols using culture or PCR based methods are standardized and validated by many national and international organizations as guidelines in public health laboratories for detection and enumeration of *E. coli* O157:H7 [10, 11]. These conventional methods are applied for the detection of *E. coli* O157:H7 all over the world due to their sensitivity to target microorganisms, efficiency, reproducibility and suitability to a wide range of food matrices, and they are considered as more reliable options to confirm the obtained results of the presence of the pathogens. However, despite the advantages and wide acceptance of these conventional methods, there are several drawbacks such as requiring certain instrumentations, consuming large amounts of media which also leads to the generation of large amounts of biological waste, time-consuming, suffering from the interference of complex food matrices, and requiring trained technicians to carefully prepare samples, correctly carry out the test procedures, and interpret the obtained results, which make them less satisfying to prevent infections of *E. coli* O157:H7. There are also other detection methods which are less common, such as ATP bioluminescence [12], flow cytometry [13], and solid-phase cytometry [14]. Recent development for rapid detection

methods have focused on the improvements on specificity, reliability, feasibility in different environment, rapid technique, miniaturization, and low-cost [15].

Please insert Figure 1 near here

The acknowledged world's first biosensor was fabricated by Clark and Lyons for the detection of glucose using immobilization of glucose oxidase on the electrode and the electrochemical detection of hydrogen peroxide generated in the reduction-oxidation of glucose [16]. Since then, incredible innovations have been integrated into the development of biosensors using novel approaches to improve their sensing performance. The term "biosensor" refers to an analytical device which is comprised of a biological receptor that can specifically recognize and capture the target analyte, a physical or chemical transducer that convert the biological and/or chemical event into a quantifiable and analyzable signal (Fig. 1) [17-21]. As shown in Fig. 1, the biological receptors used in a biosensor can be antibodies (ABs), aptamers, enzymes, ssDNA/RNA probes, bacteriophage, and more. Furthermore, based on the transduction mechanism, biosensors can be classified into optical (including light adsorption/reflection, surface plasmon resonance (SPR), fluorescence, luminescence, and optical fiber), mass or mass-sensitive (such as quartz crystal microbalance (QCM), surface acoustic wave, magnetoelastic, and cantilever), electrochemical (including amperometric/voltammetric, potentiometric, impedimetric, and conductometric), calorimetric, and others. Optical biosensors are based on the optical properties of the samples or the biological labels that are affected by the interaction between the analyte and the biological recognition element. Electrochemical biosensors rely on the attachment of labels (most enzymes) or the interaction of bioreceptors and bacterial cells, which can alter the electrical parameters like current, potential, or impedance at the surface of electrodes. Piezoelectric biosensors are mass-sensitive detectors based on a quartz-crystal resonator oscillating at a fundamental, surface-geometry-dependent frequency. It can establish a quantitative relationship between the mass changes on the crystal surface and the resonant frequency of the crystal. Based on a literature search under the key words of "biosensors" and "*Escherichia coli* O157:H7" on the EBSCO*host* research databases, the number of recent publications (from 2000 to 2015) using different transducers in the development of biosensors for the detection of *E. coli* O157:H7 have been shown in Fig. 2(a). Optical and electrochemical biosensors are still the transduction techniques studied the most for the detection of *E. coli* O157:H7.

Please insert Figure 2 near here

Among all the biosensor-based methods that have been established over the past fifteen years, electrochemical biosensors are regarded as one of the most promising directions to detect foodborne pathogens and grow rapidly (Fig. 2(b)). Electrochemical biosensors can be broadly classified into label-dependent and label-free techniques according to their detection strategies. Label-dependent methods apply labels (such as enzymes, conductive polymers, metal particles, and more) to specifically tag the target analyte, whereas the label-free techniques are based on the attachment of bacterial cells onto the electrode surface to induce measurable changes in electrical parameters [22]. Based on the electrical parameters being measured, electrochemical biosensors can be further divided into sub-categories of amperometric, potentiometric, conductometric, and impedimetric.

There have been many comprehensive review articles published aiming to illustrate the latest states on biosensors for the detection of foodborne pathogen [19, 23-26], electrochemical biosensors for virus detection [27] and toxins [28], but a review on electrochemical biosensors for detection of *E. coli* O157:H7 is missing. Therefore, this review is designated to summarize some of recent publications (from 2000 to 2015) using electrochemical biosensors to specifically detect *E. coli* O157:H7 as the model pathogenic bacterium, and divides these papers based on the sensing strategies and measured electrical parameters. The discussions include sensing configurations, sensing performance, strength and weakness of the methods, and the future trends. This survey is hoped to illustrate a broad and comprehensive understanding of electrochemical biosensors for the detection of foodborne pathogens.

2. Electrochemical biosensors for the detection of *E. coli* O157:H7

Electrochemical biosensors are intensively studied and well developed for the detection of foodborne pathogens. Some advantages of electrochemical biosensors over others include comparable sensitivity, fast response, possibility to operate in turbid solution, and possibility to be miniaturized. Furthermore, due the current state-of-the-art techniques used in the fabrication of electronics, the electrochemical biosensors can be integrated into one simple device, automated, and cost low [17, 19, 25, 28, 29]. On the other hand, to apply electrochemical biosensors for the detection of foodborne pathogens, there are still some drawbacks need to be overcome. Difficulties due to complex food samples are the most concerned. The bacteria are highly unlikely distributed in/on foods in the pattern of uniformity. Therefore, it makes the direct use of electrochemical biosensors for the detection of pathogenic bacteria

very hard without appropriate sample collections and preparations. Moreover, the complexity of food matrices can vary based on the content (proteins, fat, carbohydrates, chemicals, etc.), the formality (solid, liquid, gel, or other types), and the viscosity, which can greatly affect the performance of biosensors [30]. The specificity of the electrochemical biosensors is also concerned, especially to the bacteria with no health risks but can interfere with the specific recognition by the bioreceptors in the sensors. Therefore, recent research in the development of electrochemical biosensors for the detection of foodborne pathogens have focused on the improvement of aspects such as novel bioreceptors, better design of electrodes, and nanomaterials for effective electron transduction. Based on the mechanism and the electric parameters being monitored, electrochemical biosensors can be further divided into amperometric/voltammetric, potentiometric, and impedimetric/conductometric biosensors.

Please insert Figure 3 near here

Amperometric/voltammetric biosensors monitor the changes in current or potential that caused by the oxidation or reduction of the electrochemically active analyte in the electrochemical system. The Cottrell equation can be used to express the relation between the electric current and the concentration of the analyte:

$$i = nFAC_o[D/(\pi t)]^{1/2}, \quad (1)$$

where i is the current being monitored, n is the number of electrons being transferred in the redox reaction, F is the Faraday constant (96,485 C mol^{-1}), A is the area of electrode, C_o is the initial concentration of the reducible analyte, D is the diffusion coefficient of the reducible analyte in the media, t is the time elapsed since the potential is applied. The term "amperometry" refers to the current is measured when the potential is fixed at a constant value, and the term "voltammetry" refers to the current is measured when the applied potential is changed under controlled variations [31]. This type of electrochemical biosensors is commonly fabricated on the basis of an enzyme system (Fig. 3(a)). The electrochemically inactive substrates in the solution are catalyzed by the enzyme into electrochemically active products that can have the electron transfer at the surface of the working electrode, or the electrons are transferred from the enzyme to the electrode through direct contact or reversible oxidizing reagents (mediators) [28, 31]. The performance of amperometric/voltammetric biosensors are mainly restricted by the interferences of other electrochemical active compounds that have similar redox potentials to the analyte of interest.

Due to the use of enzyme, this type of biosensors is also limited by the saturation kinetics, the denaturation in the process, and the stability of the applied enzyme.

Potentiometric biosensors operate using a high voltmeter to measure the electrical potential difference or electromotive force between the working and reference electrodes. The electrochemical process is non-faradic with no, or negligible, net current flow in the electrochemical cell, and the potential difference is generated due to the accumulation of charge density at the surface of working electrode. A potentiometric biosensor is commonly comprised of an ion perm-selective outlayer and a bioactive material (enzymes). The measurement is based on the Nernest equation which describes a logarithmic relationship of the concentration response to the potential:

$$E = E_0 + [RT/(nF)] \ln a, \quad (2)$$

where E is the potential for the measurement, E_0 is the standard potential when a = 1 mol l^{-1}, R is the gas constant, 8.314 J mol^{-1} K^{-1}, T is the absolute temperature in K, n is the number of electrons transferred in the electrode reaction, F is the Faraday constant, and a is the relative reactivity of the interested ion based on the concentration at the anode and the cathode [33]. Potentiometric biosensors are the least common type of electrochemical biosensors in the field of foodborne pathogen detection [19, 28], but there are still some configurations have been proven to be feasible. Ion-selective field effect transistors (ISFETs) and light-addressable potentiometric sensors (LAPSs) are the two major types of potentiometric biosensors that have been studied. ISFETs response to specific ions (H^+, K^+, Cl^-, etc.) by applying an electric field to create regions of excess charge in a semiconductor substrate and using an ion-selective membrane to cover the gate insulator to allow the ion of interest to pass (Fig. 3(b)) [34]. Studies on ISFET-based biosensors for the detection of pathogens have indicated poor limits of detection (LODs) and device stability due to the incompatibility between most biomolecular immobilization protocols and ISFET fabrication technology [4, 25]. On the other hand, development on the LAPSs has proved this type of potentiometric biosensors are feasible for the detection of foodborne pathogens [35]. LAPS is based on the application of transient illumination using an intensity-modulated light source like light-emitting diodes (LEDs) to induce a transient photocurrent to an insulated n- or p- doped semiconductor thin film which is in contact with an electrolyte. The magnitude of the induced photocurrent depends on the potential applied to the semiconductor plate. There is a commercial available LAPS device, the Threshold® Immunoassay System for the detection of *E. coli* O157:H7 in food samples [36].

Impedimetric/conductometric biosensors are probably one of the earliest types of physiochemical methods developed for the rapid detection of bacteria. This type of biosensors is commonly based the technique known as

electrochemical impedance spectroscopy (EIS) which applies a sinusoidal potential with very small amplitude to the electrochemical system and measure the resulting current over a range of varying excitation frequency. The obtained data can be fitted into an equivalent circuit to generate valuable electrochemical parameters, including charge transfer resistance, double layer capacitance, bulk solution resistance, and inductive reactance for interpretation. The fundamental relationship in EIS can be expressed as following equation:

$$Z = R + jX = R + j(X_L - X_C), \quad (3)$$

where Z is the complex impedance which is the sum of the real and imaginary impedance components, R is the resistance, X is reactance, XL is the inductive reactance which is normally negligible in a biological system, XC is the capacitive reactance which equals to $1/(2\pi fC)$ where f is the frequency and C is the capacitance. As shown in Fig. 3(c), the working principle of impedimetric/conductometric biosensors is based on either the immobilization of bioreceptors on the surface of the working electrode and directly measure the impedance change caused by the capture of bacteria, or the application of labels (enzymes or conductive materials) or the microbial metabolism to produce ions in the media which increase the conductance or capacitance thereby decreasing the impedance of the media [38]. Recent years, a vast of studies have been done on the impedimetric biosensors using the direct detection strategy because the labels are no longer necessary in this setup. Thus, the detection time is greatly shorten, the detection process becomes simpler and less washing steps are involved. The limitations of impedimetric biosensors are on the complicated immobilization procedures which can greatly affect the reproducibility and regenerability of the fabricated sensors, and the LODs using EIS for pathogen detection are still not low enough [4].

More information regarding the published articles using electrochemical biosensors for the detection of *E. coli* O157:H7 are discussed with details in the following sections. The papers are divided based on the detection strategies used to fabricate the biosensors, namely the label-dependent and the label-free techniques, and are also listed in Table 1.

2.1 Label-dependent electrochemical biosensors

Please insert Figure 4 near here

To design an electrochemical biosensor, there are generally two detection strategies, label-dependent and label-free techniques, to convert the biological recognition event into electrical signals. First, label-dependent electrochemical biosensors employed an indirect detection which is usually based on the labels of biological or physiochemical molecules such as enzymes, metallic nanoparticles, conductive polymers, and others on the target bacterial cells to induce the electrical changes for measurement (Fig. 4). There was a voltammetric method using enzyme-linked immunomagnetic electrochemistry that sandwiched the target bacteria, *E. coli* O157:H7, between the immunomagnetic beads (IMBs) and the enzyme-conjugated antibodies [39]. The captured bacteria were adsorbed onto a magnetized graphite electrode in a multiwell plate, and the LOD was achieved at 3.3×10^3 cfu ml^{-1} in Tris-saline and 1.5×10^3 cfu ml^{-1} in spiked buffered apple juice. Another amperometric biosensor was fabricated based on an indirect ELISA with double antibodies and a disposable AuNPs-modified SPCE [32]. The Ferrocenedicarboxylic acid (FeDC) modified AuNPs on the SPCEs had significantly improved the sensitivity of the fabricated biosensor, and achieved the LOD for the detection of *E. coli* O157:H7 at 6×10^2 cfu ml^{-1} in PBS and 5×10^3 cfu ml^{-1} in milk. Because the biological recognition events in this type of electrochemical biosensor are converted to electric signals through the labels, different labels can be employed to achieve simultaneous detection of multiple bacteria. Viswanathan and others reported an electrochemical immunosensor to simultaneously detect three pathogens, *E. coli* O157:H7, *Salmonella*, and *Campylobacter* [40]. They fabricated the biosensor based on the immobilization of a mixture of anti-*E. coli*, anti-*Salmonella*, and anti-*Campylobacter* ABs functionalized multiwall carbon nanotube-polyallylamine (MWCNT-PAH) onto the surface of screen-printed electrodes (SPEs). After the target bacteria were captured, specific nanocrystals conjugated respective ABs (AB-*E. coli*-CdS, AB-*Salmonella*-CuS, and AB-*Campylobacter*-PbS) were used to label the three bacteria and released different metal ions for the electrochemical measurement. The constructed immunosensor showed LODs of three selected bacteria at 0.8×10^3 cfu ml^{-1}, 0.4×10^3 cfu ml^{-1}, and 0.4×10^3 cfu ml^{-1}, respectively. Metallic nanoparticles are also used as powerful labels for the fabrication of electrochemical biosensors. A most recent amperometric biosensor was developed for the detection of *E. coli* O157:H7 in minced beef and water using electrocatalytic gold nanoparticle tags [41]. The way for fabricating this biosensor was to use a magnetosandwich assay between the AB-conjugated magnetic beads, the target heat-killed *E. coli* O157:H7, and second AB-conjugated AuNPs. The AuNP tags catalyzed hydrogen

evolution reaction which was used for quantitation of the target bacteria. The LODs of 148, 457, and 309 cfu ml^{-1} were achieved in buffer, minced beef, and tap water samples, respectively, within approximately 1 h.

Please insert Table 1 near here

The advantage of using label-dependent strategy is that the labels usually provide effective amplification of the electrical signals because the labels are chosen because they can offer an efficient and specific electron transfer pathway with a high electron transfer rate. Therefore, this strategy can yield lower LODs and improve the specificity of the electrochemical biosensors. Moreover, the conversion of the biological recognition events in these biosensors depends on the labels. The formation of the target-label interaction can happen anywhere, in the solution or on the electrode surface, which allows to improve the efficiency of capturing target bacteria in the complex food matrices by using distinguish carriers such as magnetic beads to directly separate the bacterial cells. On the other hand, the disadvantages of this type of electrochemical biosensors also come from the labelling procedures which prolong the detection time as well as add more complexity in the operation.

2.2 Label-free electrochemical biosensors

Please insert Figure 5 near here

The second type of strategy employed by the electrochemical biosensors is the label-free direct detection of the target bacteria based on the immobilization of bioreceptors on the surface of the electrodes (Fig. 5). It is reported the membranes of natural bacteria cells (with a thickness of 5 to 10 nm) show a capacitance of 0.5 to 1.3 μF cm^{-2} and a resistance of 10^2 to 10^5 Ω cm^2 [42]. Therefore, the attachment of the bacterial cells can directly induce the change in the electrical parameters. Maalouf *et al*. developed an electrochemical impedance biosensor for the detection of *E. coli* based on a mixed self-assembled monolayer of thiol-neutravidin on the gold electrode [43]. It achieved the LODs of 10 cfu ml^{-1} for the whole bacterial cell and 10^3 cfu ml^{-1} for the lysed *E. coli*, which were much lower comparing to 10^7 cfu ml^{-1} when using SPR method with the same electrode modification and antibodies. Interdigitated array microelectrodes (IDAM) were also used to develop impedance biosensor for the detection of *E. coli* O157:H7 in ground beef [44]. The ABs-conjugated magnetic nanoparticles were used in this method to separate the target bacteria from the food matrix, and attracted and concentrated the bacteria with a magnet to the active layer

of the IDAM. The LODs of this biosensor for the detection of *E. coli* O157:H7 were 7.4×10^4 cfu ml^{-1} and 8.0×10^5 cfu ml^{-1} in pure culture and in ground beef, respectively, with the detection time of 35 min. A quartz crystal Au electrode-based impedimetric immunosensor was developed for the detection of *E. coli* O157:H7 [45]. This immunosensor used a simple facile modification of the quartz crystal Au electrodes with protein A, followed by the functionalization using anti-*E. coli* ABs. The relatively large surface of the quartz crystal Au electrodes used in this study allowed more bacterial cells captured thus increased the impedance change. This label-free immunosensor showed the LOD for *E. coli* O157:H7 at 10^3 cfu ml^{-1} and with the detection time of less than 10 min. Another potentiometric biosensor was studied for the detection of *E. coli* CECT 675 as a surrogate for the pathogenic *E. coli* O157:H7 in a direct and simple label-free manner [46]. This biosensor used aptamers covalently immobilized on the single-walled carbon nanotubes (SWCNTs) which were deposited on the glassy carbon electrode (GCE). After a filtration pre-treatment step to separate the target bacteria from the interfering food matrix, this method could detect as low as 6 cfu ml^{-1} in milk or 26 cfu ml^{-1} in apple juice.

The immobilization strategy has been identified as the most important procedure for label-free biosensors. Many kinds of the novel materials or techniques have been studied to improve the sensitivity, stability, regenerability, and reproducibility of biosensors employing label-free strategy. A capacitive immunosensor was developed based on the label-free strategy for the detection of *E. coli* O157:H7 [47]. This immunosensor was fabricated using SAMs of 3-mercaptoprotionic acid (MPA) to immobilize the anti-*E .coli* ABs onto a quartz crystal Au electrode. The change in capacitance from the attachment of the bacteria was measured and analyzed using EIS. The constructed capacitive immunosensor could discriminate *E. coli* O157:H7 as low as 10^2 cfu ml^{-1} in PBS, 10^3 cfu ml^{-1}, 10^3 cfu ml^{-1}, and 10^4 cfu ml^{-1} in milk, spinach, and ground beef, respectively. An amperometric immunosensor based on the formation of an ordered, oriented, compact, and stable SAM of alkanethiol 11-amino-1-undecanethiol hydrochloride (AUT) was developed for the detection of *E. coli* O157:H7 [48]. The monolayer of AUT with rich amine functional groups allowed attachment of massive AuNPs. Then the active surface of the gold electrode with AUT/AuNPs was further enhanced by multilayer films comprised of chitosan-multiwalled carbon nanotubes-SiO_2/thionine (CHIT-MWCNTs-SiO_2@THI) nanocomposites and AuNPs. The layer-by-layer assembly not only improved the stability of the immobilization on the electrode surface, but also increased the sensitivity of the biosensor due to the rich quantity of THI mediator immobilized on the electrode. This label-free amperometric immunosensor was able to detect heat killed *E. coli* O157:H7 as low as 250 cfu ml^{-1} within less than 45 min. The

graphene, owing to its unique structure of single-atom-thick planar sheet of carbon atoms, has extraordinary electrical properties and was used to develop a label-free immunosensor to detect *E. coli* [49]. The graphene film used to fabricate the conductometric biosensor was grown on the quartz substrate by chemical vapor deposition and functionalized with anti-*E. coli* ABs. The change in conductance was distinguishable from the blank samples after exposure to the target bacteria at 10 cfu ml^{-1} for 30 min.

Recent years, label-free impedimetric biosensors for the detection of *E. coli* O157:H7 have been intensively studied. A nanoporous membrane was applied to construct an impedimetric immunosensor for the label-free detection of *E. coli* O157:H7 in milk [50]. This method modified an alumina nanoporous membrane by using hyaluronic acid (HA) to reduce the non-specific binding and allow the successful immobilization of ABs on the nanoporous membrane. The ionic impedance of the electrolytes flowing through the membrane was monitored by EIS and increased due to the blockage from bacteria attachment on the nanoporous membrane. The LOD was found to be 10 cfu ml^{-1} in buffer and approximately 84 cfu ml^{-1} in whole milk. There was a highly sensitive impedimetric biosensor developed for the detection of *E. coli* O157:H7 [51]. To fabricate this biosensor, the gold electrode surface was first immobilized with SAM of 16-mercaptohexadecanoic acid (MHDA), and then the free end of MHDA was chemically attached with carboxyl groups to allow further functionalization by ABs. The density of the ABs attached on the surface of electrodes was estimated to be 476 ± 16 ng cm^{-2}. The LOD of this biosensor was achieved at 2 cfu ml^{-1}. Another label-free impedimetric immunosensor was developed for the detection of *E. coli* O157:H7 based on a free-standing reduced graphene oxide paper (rGOP) which was surface modified with AuNPs for further functionalization with ABs [52]. After incubation with the target bacteria for 30 min, the impedance was positively related to the increase of the concentration of the bacteria, and the LODs of this immunosensor was 1.5×10^2 cfu ml^{-1} in redox buffer, and 1.5×10^3 cfu ml^{-1} and 1.5×10^4 cfu ml^{-1} in spiked pre-treated cucumber and ground beef rinsing solutions, respectively. The graphene modified SPEs which have more effectiveness on the electron transfer and increased active area were used to construct a disposable impedimetric biosensor to determine the presence of *E. coli* O157:H7 [53]. The biosensor was fabricated using electrochemically reduced graphene oxide to form the graphene film on the surface of the SPEs, and followed by electrochemical deposition of AuNPs for further simple and efficient physical adsorption of ABs. The change in charge transfer resistance was positively related to the concentration of the target bacteria, and the LOD of this developed impedimetric biosensor was 1.5×10^3 cfu ml^{-1} with the detection time of 30 min. The most recent development of an indium tin oxide (ITO) based label-free

impedimetric biosensor claimed to be able to detect *E. coli* O157:H7 at a very low LOD of 1 cfu ml^{-1} [54]. The biosensor was fabricated based on a robust surface functionalization using silane monolayers on the ITO substrate and followed by ABs immobilization. The binding efficiency of the ABs was increased by 30% which allowed for more favorable capture of the target bacteria. This method could be a very powerful platform for the detection of foodborne pathogens.

The advantages of using label-free strategy to construct electrochemical biosensors are obvious. The nature of label-free strategy allows the constructed sensors to have a straightforward and simple detection process with relatively less time consumed. The direct detection of the target bacteria also allows the integration of the label-free electrochemical biosensors into one test chip. On the other hand, the disadvantages of label-free strategy for foodborne pathogens detection are apparent as well. First, the elimination of additional labels makes the label-free electrochemical biosensors lack of additional signal amplification. As stated previously, the electric properties of individual cell are at very small values. Therefore, in order to reach a detectable electrochemical signal, it requires either enough number of bacterial cells captured (higher LOD), or to use complicated immobilization methods to enhance the detector's capability of sensing the small signal. Second, the incubation of the target bacteria with the bioreceptor-immobilized electrodes has also restricted the label-free biosensors to reach lower detection limit. When using the label-free biosensors, the target bacteria are captured upon the contact with the bioreceptors on the surface of electrodes. However, it only depends on the mobility of the bacteria because the common operation for incubation process is to put the liquid sample on the active area of the electrode and let it keep stationary without any mixing. Therefore, there is high possibility that some of the bacteria cannot be captured and contribute to the electrochemical measurement. Last but not least, to fabricate an effective label-free electrochemical biosensor, the immobilization of mediators or conductive or semi-conductive materials onto the surface of electrodes is extremely important. Considering the complex process most of the immobilization methods employed, the cost of making these biosensors is increased and the possibility of mass producing these sensors is reduced. Furthermore, the immobilization procedures involved in the fabrication of the label-free electrochemical biosensors usually include the rough cleanness steps and the biochemical attachment of compacted functional molecules on the surface of electrodes. The cleanness of the electrodes may include physical abrasion using alumina powder, chemical corrosion using either strong acid such as hydrochloride acid or basic such as sodium hydroxide. All these cleanness procedures could have damaging effect to the surface of the electrodes, especially delicate ones like interdigitated

array microelectrodes. And the chemical immobilization steps include covalent or non-covalent binding of functional groups such amine or carboxyl for the further adsorption of ABs, mediators, conductive or semi-conductive materials. Such strong bond between the functional groups and the electrode provides necessary stability and high efficiency on electron transfer, whereas it usually causes difficulties for the regeneration and reuse of the electrodes. All of above advantages and disadvantages have made the electrochemical biosensors using label-free strategies become very appealing yet demanding for further improvement. In the most recent work by our group, a concept of using a synthesized bifunctional polymeric nanocomposites (PNCs) was used to fabricate an electrochemical biosensors to detect *E. coli* O157:H7 [55]. The synthesized PNCs were served as the carrier to isolate the target pathogens as well as the electrochemical signal generator/amplifier, which possessed advantages combining both the label-based and label-free detection strategies. This concept could point out a new direction to fabricate highly efficient electrochemical biosensors to detect the food pathogens.

3. Recent trends in the development of electrochemical biosensors for *E. coli* O157:H7

Significant efforts have been focused on the detection of *E. coli* O157:H7 in research because its important role as the sanitary indicator and the zero-tolerance policy regarding its presence in foods [10, 56]. The development of biosensors for whole bacterial cells is challenging due to the much larger size of the analyte than regular biochemical molecules, and also the surface of the bacterial cells are comprised of various epitopes that allow nonspecific interactions with the impurities or the sensor surface. An ideal biosensor should include at least some key properties as follows (Table 2). Depending on the site of interests (at home, at clinics, in a lab, at the production line, wastewater site, *etc.*), the configuration can be vary, but the core demanding for the biosensor should be almost the same [23]. Recent years, with various advanced techniques and materials being applied in the development of biosensors, the detection systems, which offered increased sensitivity and specificity as well as the integration of miniaturized and automated instrumentations, have directed toward the implementation of a portable platform for rapid detection of foodborne pathogens.

Please insert Table 2 near here

3.1 Applications of nanotechnology in electrochemical biosensors for improvements in sensing performance

The most convenient biological detection methods are taking advantage of selective recognition between receptor-ligand interactions. The construction of biosensors generally use this same strategy. The nanomaterials can offer much more improvements in sensitivity, specificity, and speed compared to other biochemical methods based on the microscale materials, because the nanomaterials can possess unique electrical properties, high surface-to-volume ratio, and easily functionalized surfaces.

One serious problem in the fabrication of electrochemical biosensors for detection of *E. coli* O157:H7 or other pathogens is to deal with the various interferences in the complex food matrices. Even though there are techniques like filtration or centrifugation being used to isolate the bacteria of interest from the food samples, the most often and efficient isolation method right now is the well-known immunomagnetic separation (IMS). In IMS, magnetic particles are applied as the carriers with corresponding ABs and a magnet is used to separate the captured target bacterial cells from the food matrix prior to detection. The nanoscale magnetic particles are extremely useful due to their high surface-to-volume ratio which facilitates the high capture efficiency of large microbial analyte. The captured analyte can later be subjected to concentration and measurement. There was one study reported the capture of *E. coli* O157:H7 in ground beef using streptavidin-coated immunomagnetic beads (IMBs) [57]. The study pointed out that under the optimal parameters the capture efficiency showed at least 94% of *E. coli* O157:H7 in the range of 1.6×10^1 to 7.23×10^7 cfu ml^{-1} captured with an immuno-incubation time of 15 min without any enrichment, and no significant interference from other non-target bacterial species. In one of our previous work, an impedance immunosensor was developed also based on IMS to separate and concentrate *E. coli* O157:H7 from food matrix, and the result showed over 90% capture efficiency in this study (Fig. 6) [58].

Please insert Figure 6 near here

The recognition elements of biosensors are considered expensive and vulnerable if they are biologically produced. For instance, for antibody or cell based biosensors, the antibodies or the engineered cells are normally produced in tissue culture or animals, which can be costly and also time-consuming to develop them with high specificity. Furthermore, for such biological elements, they are also fragile to environment and required special preservation such as refrigeration. Therefore, there has been great interest in the development of artificial or synthetic recognition elements to replace those from biological hosts. The molecularly imprinted nanomaterials are

one of the most interesting direction in the design of synthetic bioreceptors [59]. The imprinting process first involves the arrangement of polymerizable monomers with template-specific binding sites to surround the templates or imprint molecules, and then proceeds to polymerization of monomers with a cross-linker. After the extraction of the templates after polymerization, the result 3-D polymers should have imprinted cavities inside that can selectively bind to the template molecules. A broad categories of imprinted nanomaterials have already been developed, including imprinted nanoparticles [60], imprinted nanocomposites [61], and imprinted hybrid materials [62]. The molecularly imprinting technique is mostly applied to detect chemicals or small molecules, but there have been a few studies targeting whole bacteria cells [63-66]. The achieved LODs for the target bacteria were in the range of 10^3 to 10^4 cfu ml^{-1}.

The transducer in an electrochemical biosensor plays a very essential role in the detection process. There have been a broad spectrum of transduction methods employed for the detection of foodborne pathogens over the past decade. Even though new techniques constantly being developed in transducers for better performance of biosensors, the transduction mechanisms can be divided into two categories as stated in section 2, the label-dependent and the label-free ones, which have different requirements for the high efficient transducers. Currently, the nanofabrication techniques can shred the size of a sensing probe to the level where the bacterial cells are at, improving the sensitivity and lowering the LOD enormously.

As the label-free methods for the fabrication of electrochemical biosensors, nanomaterials are particularly helpful on the immobilization of bioaffinity agents. The early-stage biosensors usually immobilized enzymes or antibodies through the reaction with cross-linkers such as glutaraldehyde and blocked the free surface by blocking agents such as bovine serum albumin (BSA). This type of immobilization methods commonly result in random distribution of the bioreceptors which utilize only proportion of the active area of electrodes and also caused denaturation of the enzymes or antibodies [67]. Nanoparticles have provided means to overcome such problems by allowing high loading capacity with controlled orientation of bioreceptors attachment. The most widely applied example of using nanoparticles to facilitate the bioaffinity reagents immobilization is with gold nanoparticles which can be easily amended by thiol bonds. Relatively simple chemical synthesis of gold nanoparticles on various surfaces makes it suitable for surface immobilization and functionalization to construct biosensors. Moreover, the high conductivity of gold nanoparticles can improve the electron transfer rate to the electrode surface and hence increase the sensitivity of the assay [68].

For label-dependent methods, metallic nanoparticles and conductive polymers are emerging as powerful platforms for signal amplification due to their excellent conductivity. One study done by Muhammad-Tahir and Alocilja [69] used antibody conjugated conductive polyaniline to label the target *E. coli* O157:H7. The construction of the conductometric biosensor was based on a lateral flow system that lead the liquid sample to move from the application pad to conjugation pad and then to the capture pad. On the capture pad, the polyaniline-labeled bacterial cells were captured by primary antibodies immobilized between two electrodes. The amount of labelling polyaniline existed in the solution changed the conductance, and the LOD of this biosensor was achieved at 79 cfu ml^{-1} with a detection process of 10 min. When the polymers are used to form nanoscale spherical or granular nanoparticles, they exhibit excellent properties such as good biocompatibility and functionality which are very suitable for candidates as carriers to enhance the probe loading. There was a poly (styrene-*co*-acrylic acid) microspheres with diameter of 338 nm prepared by Pinijsuwan *et al*. [70]. The latex spheres were modified with layer-by-layer assembly of avidin-labeled AuNPs which showed two orders of magnitude increase in the amount of Au atoms delivered per nanocomposite microsphere. This as-synthesized nanocomposite was immobilized on an electrode surface and capture *E. coli* by using *E. coli* specific DNA probes via DNA hybridization. A single DNA hybridization signal could be translated into hundreds of AuNPs release by using this strategy.

3.2 Lab-on-a-chip techniques in the miniaturization of electrochemical biosensors for detection of E. coli O157:H7

As one of the desired properties, the portability of an electrochemical biosensors is of great importance to realize in-field detection of foodborne pathogens. Even though the definition of a "portable" electrochemical biosensor can vary, an ideal portable device should be a single unit that is possibly operated with one hand, weighted less than one kilogram, battery-supported, and integrated with its own microprocessor, display screen and control system. Recently, the concept of lab-on-a-chip has been suggested as an appropriate solution to construct portable and real-time electrochemical biosensors for detection of foodborne pathogens [71]. Lab-on-a-chip refers to a device that combines multiple laboratory functions into one small chip with dimensions at millimeters or centimeters level. This is also known as the microfluidic devices that fabricate a network of channels and wells etched onto glass, silicon, or polymer chips, or utilize the latest 3-D printing technique to directly print out the chips [72]. Such microfluidic devices can precisely control the small volume of fluid samples through pressure or electrokinetic forces and enable the integration of laboratory procedures like mixing, diluting, staining, and detection operated in a

manner of automation. Lab-on-a-chip technique brings electrochemical biosensors with advantages such as easy-to-use, miniaturization, small volume of samples consumption, standard and mass production, and automation.

Electrochemical lab-on-a-chip biosensors have been successfully fabricated for detection of *E. coli* O157:H7 in different food samples. Varshney *et al*. [73] developed an electrochemical impedance microfluidic biosensor that the detection chamber was fabricated by integrating an interdigitated array microelectrode into a poly(dimethylsiloxane) (PDMS) microchannel (Fig. 7). The detection chamber was 6 mm × 0.5 mm × 0.02 mm that could handle 60 nl of samples. *E. coli* O157:H7 spiked on ground beef was separated and concentrated by using antibody-conjugated magnetic nanoparticles and then injected into the microfluidic flow cell for impedance measurement. This label-free impedance microfluidic biosensor achieved the detection limit of 1.2×10^3 cfu ml^{-1} in ground beef with the detection time of 35 min. Latest development of microfluidic device integrated with nanoporous membranes demonstrated the simultaneous detection of *E. coli* O157:H7 and *Staphylococcus aureus* from the mixed samples [74]. Two separated testing chambers with same sample inject channel but different measurement chambers were fabricated with two layers of polymeric polyethylene glycol (PEG) with nanoporous membranes assembled in between. Then the membranes were functionalized with anti-*E. coli* O157:H7 and anti-*S. aureus* antibodies, respectively. Upon the binding of target pathogens on the corresponding nanoporous membranes, the electrolytes were blocked from passing through some of the nanopores which increased the magnitude of impedance signal. The LODs of this microfluidic nanopore-based impedance biosensor achieved 10^2 cfu ml^{-1} for both pathogens in the mixed samples.

Please insert Figure 7 near here

3.3 Techniques to fabricate low-cost electrochemical biosensors

Screen-printing technique is one of the most promising methods to produce flexibly designed, inexpensive, and reproducible electrodes that can find various applications in the fabrication of disposable and portable electrochemical biosensors [75]. This technology is based on the layer-by-layer of desired ink deposited onto a solid substrate by using a screen or mesh. The most common ink materials are silver and carbon, others like gold and platinum can also be used but with higher cost. Based on the experimental requirement, different ink formulations, the types of inks, the size of the design, the loading of ink materials, and the printing or curing conditions can strongly affect the sensitivity or selectivity and overall analytical performance of the fabricated biosensors [76, 77].

Furthermore, there have been an extensive spectrum of modification methods to immobilize different substances like metals, enzymes, polymers, electrochemical mediators, or complex mixtures onto the SPEs that can offer diverse and suitable properties for the applications.

Immunosensors based on SPEs are especially attractive for point-of-care or on-site monitoring because the SPEs can allow the miniaturization of sensors, low cost, and to be disposable. There have been several studies developed SPE-based electrochemical immunosensors. Self-assembling technique was used by Escamilla-Gomez *et al*. [78] to compare two immobilization configurations on the gold SPEs (AuSPEs) for the construction of a label-free electrochemical impedance immunosensor for *E. coli* detection. The first one used homobifunctional cross-linker to immobilize the anti-*E. coli* antibodies, and the other one was based on the thiol-gold interaction to immobilize the thiolated antibodies onto the surface of AuSPEs. The electrochemical impedance spectroscopy was used in the presence of a redox probe. The immunosensor based on the thiolated antibodies achieved much better performance with a LOD of 10 cfu ml^{-1} in *E. coli* spiked river and tap water samples and an assay time of 1 h. Another lectin-based label-free impedance immunosensor using AuSPEs was reported for the detection of microorganisms [79]. The AuSPEs were successively incubated with biotinlated ConA and *E. coli* in solution. The selective interaction of ConA with the carbohydrate components on the surface of the bacterial cells allowed the formation of AuSPE-ConA-*E. coli* complex which was monitored by EIS. The LOD was achieved at 5.0 x 10^3 cfu ml^{-1} with an assay time of 1 h.

4. CONCLUSIONS, CURRENT PROBLEMS AND FUTURE OUTLOOK

As the first type of biosensors developed more than fifty years ago, electrochemical biosensors have been proved to be the most promising method to fabricate portable devices for rapid and on-site detection of foodborne pathogens. In this article, we reviewed some of the recent scientific literatures on different types of electrochemical biosensors for the detection of *E. coli* O157:H7. Comparing to the conventional methods and other biosensor counterparts, electrochemical biosensors have demonstrated advantages such as less restrictions from the sample turbidity, possible miniaturization, low cost, possible integration system, and mass production. But there are also limitations of using electrochemical biosensors based detection methods.

First, the low LOD is still the primary pursuit in the development of electrochemical biosensors to detect *E. coli* O157:H7 in food samples. Common expectation of LODs achieved by these biosensors is less than 100 cfu ml^{-1} without pre-enrichment procedures. As shown in Table 1, there are only a few reports achieved close to or less than

this criteria. The solution to this problem of sensitivity most likely lies in the development of novel materials, especially nano-scale materials. The nanomaterials can provide more cost-effective signal transduction, efficient support for biorecognition elements, and the possibility of multiplex simultaneous detection of several pathogens.

The specificity of the electrochemical biosensors for detection of *E. coli* O157:H7 is another critical issue. To achieve similar specificity to the culture-based methods, electrochemical biosensors still have a long way to go. Currently, the label-dependent electrochemical methods generally showed better specificity than that of label-free ones. Because the label-dependent methods rely on the double specific recognition elements (primary antibodies and second antibody-conjugated labels) to capture and label the target bacterial cells. The label-free methods solely depend on the recognition of primary antibodies so that the interference from non-targets like other bacteria and chemicals is problematic. Therefore, the selection of more specific bioreceptors is one of the aspects needs to be paid attention to. Some innovative biorecognition elements such as aptamers, bacteriophage, and molecularly imprinted polymers are the new trend that can help on developing high affinity, better selectivity, and more stable electrochemical biosensors for *E. coli* O157:H7 detection.

To become a commercial product, the reliability of the developed electrochemical biosensors should be checked as well. The main issues are the stability and preservation of the biological materials, the consistency of the biosensors under different working conditions, and the accuracy when dealing with the complex matrices of real foods. The bioactivity of biomaterials used to fabricate the biosensors varies based on the working environment such as temperature, pH, humidity, or storage. They can become less efficient over time due to denaturation, degradation or aggregation, which will greatly affect the performance of the electrochemical biosensors. Currently, most studies focus on the development of new materials especially conductive nanoparticles and nucleic acid based biorecognition elements to replace the traditional Abs or enzymes. These small molecules and chemicals present the advantages of low-cost, stable, and comparable efficiency to their counterparts, and become more and more common in the design of electrochemical biosensors to detect *E. coli* O157:H7 to improve the sensitivity and stability [27, 80, 81]. When facing the complex food samples, the interference of different nutrients to the detection of *E. coli* O157:H7 using electrochemical biosensors is another important issue. Even though, as mentioned in section 3.1, immunomagnetic separation can be used to isolate and concentrate *E. coli* O157:H7 from food matrix, it still prolongs the detection time. Moreover, it is not easy to integrate a magnetic separator with the portable devices.

Currently, lab-on-a-chip techniques have helped on the miniaturization and automation of the electrochemical devices in research. But it still have a long way to become practical to be applied in a commercial product.

Finally, the standardization and validation of the electrochemical biosensors to detect *E. coli* O157:H7 are presented as one important issue for the developed methods to be practiced regularly. Unlike culture- or PCR- based methods, electrochemical biosensor methods for foodborne pathogens are lack of standard protocols or guidebooks established by the official agencies (ISO, AOAC, FDA, USDA, *etc*.). Therefore, critical parameters such as LODs and detection time are determined by arguably inconsistent standards. For instance, most of the reported electrochemical biosensor methods established LODs using calculations based on signal/noise ratio of three, which the ratio is only defined as "likely to be reliably distinguishable from the blank" [82-84]. There has been other methods to estimate the LODs which may be more conservative and precise for quantitation purpose [82]. The standards used by different research groups to obtain these critical criteria have made it difficult to evaluate the performance of developed electrochemical biosensors and to compare to counterparts.

Nowadays, electrochemical biosensors for food safety are still penetrating the market very slowly due to the complicated task to design desirable biosensors with characteristics described in Table 2. In the near future, foodborne pathogen detection using electrochemical biosensors will be no-doubt to benefit from advanced techniques mentioned in section 3. There will be more stringent protocols established by officials for easy validation and consistency to develop electrochemical biosensor based methods for the detection of *E. coli* O157:H7. The electrochemical biosensors will become more sensitive, selective, precise, and be integrated into micro- or nano-devices that are easy-to-use, low cost, stable, and suitable for detection of various foodborne pathogens.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Figure 1. The schematic frame of a typical biosensor.

Figure 2. (**a**) Approximate numbers of publications using biosensor methods for the detection of *E. coli* O157:H7. (**b**) Approximate numbers of publications using electrochemical biosensors for the detection of *E. coli* O157:H7 on yearly order.

Figure 3. (a) The schematic mechanism of an amperometric biosensor. The label (commonly enzyme) accelerate the electrochemical active analyte in the solution to transfer electrons to the electrode. **(b)** The schematic mechanism of a ISFETs potentiometric biosensor. The label changes the particular ion concentration (H^+, K^+, *etc*.). The ion accumulates on the gate insulator, which produces potential different via current flows through the gate. **(c)** The schematic mechanism of an impedimetric biosensor. The impedance is increased because of the electron transfer was blocked upon the attachment of bacterial cells.

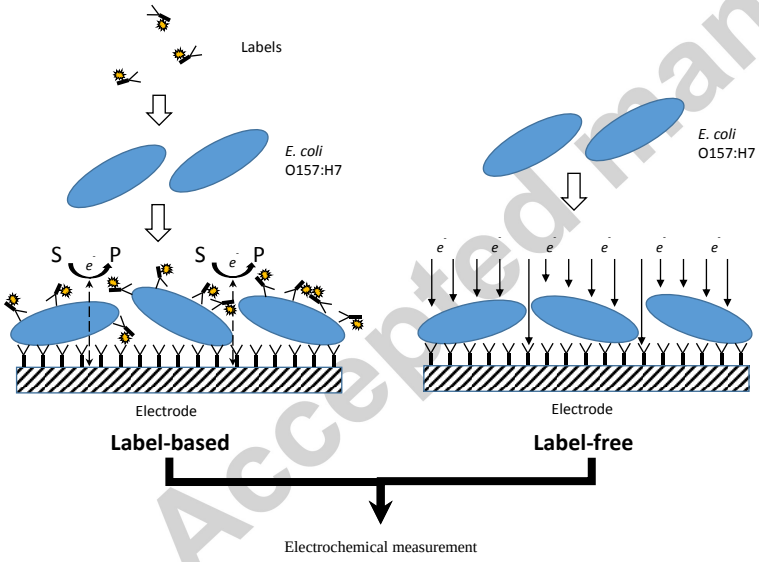
Figure 4. An amperometric biosensor based on screen-printed carbon electrode (SPCE) which was immobilized with ferrocenedicarboxylic acid (FeDC) (mediator) coated gold nanoparticles (AuNPs) and antibodies. After the capture of *E. coli* O157:H7, a horseradish peroxide (HRP) conjugated second antibody was employed to label the bacterial cells and catalyze hydrogen peroxide. The apparatus setup was shown in the bottom panel. The electrochemical system was built on a disposable testing strip [32].

Figure 5. (**a**) A label-free impedimetric biosensor for the detection of *E. coli* O157:H7. The bioreceptors were absorbed to the surface of a SP-IDME by a permanent magnet. Upon the capture of the bacterial cells, the pathway for the electron transfer at the electrode surface was blocked, and thus the impedance

was increased. (**b**) The equivalent circuit of the developed electrochemical impedance biosensor was shown. It is important for the data analysis in EIS to obtain electrical parameters like charge transfer resistance (R_{ct}) [37].

Figure 6. An electrochemical impedance immunosensor based on the IMS to separate and concentrate target pathogen, *E. coli* O157:H7, from food sample. The GOx-AB conjugates were used to label the target bacteria for electrochemical measurement [58].

Figure 7. (**a**) Glass wafer with Au IDME on the chip. (**b**) The design of microfluidic assembly with a detection microchamber, and inlet and outlet channels. (**c**) The assembled microfluidic flow cell with embedded IDME and connection wire [73].



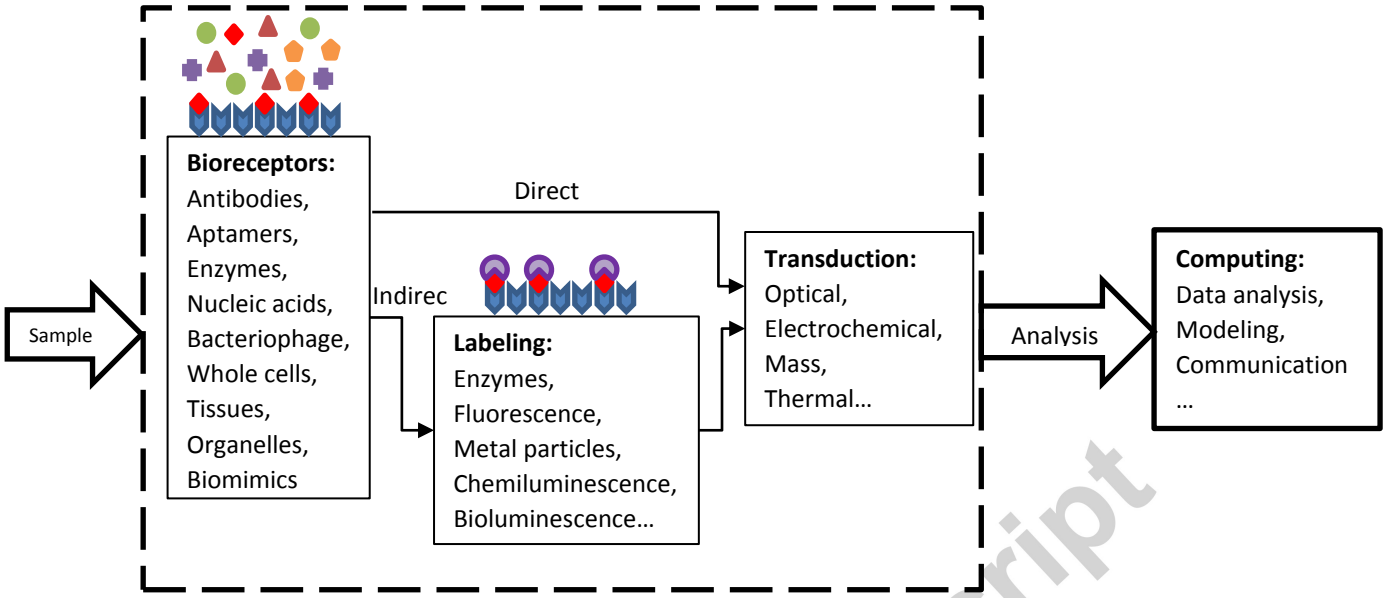


Figure 1

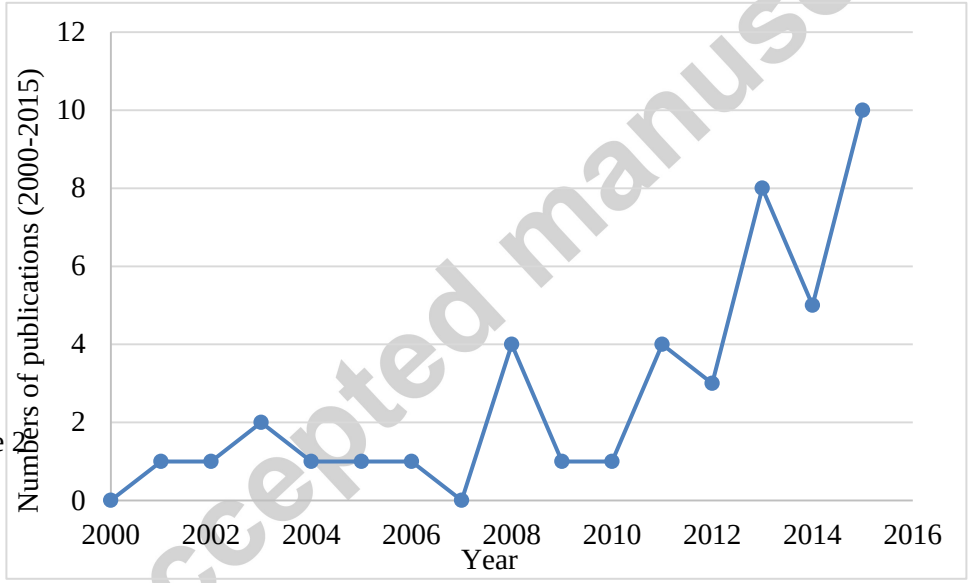


Figure 2

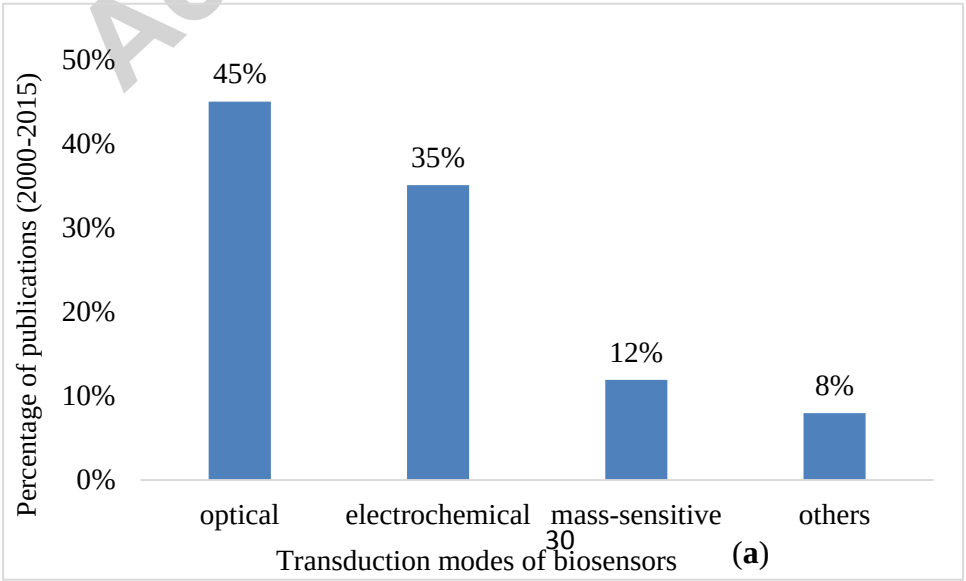


Figure 3

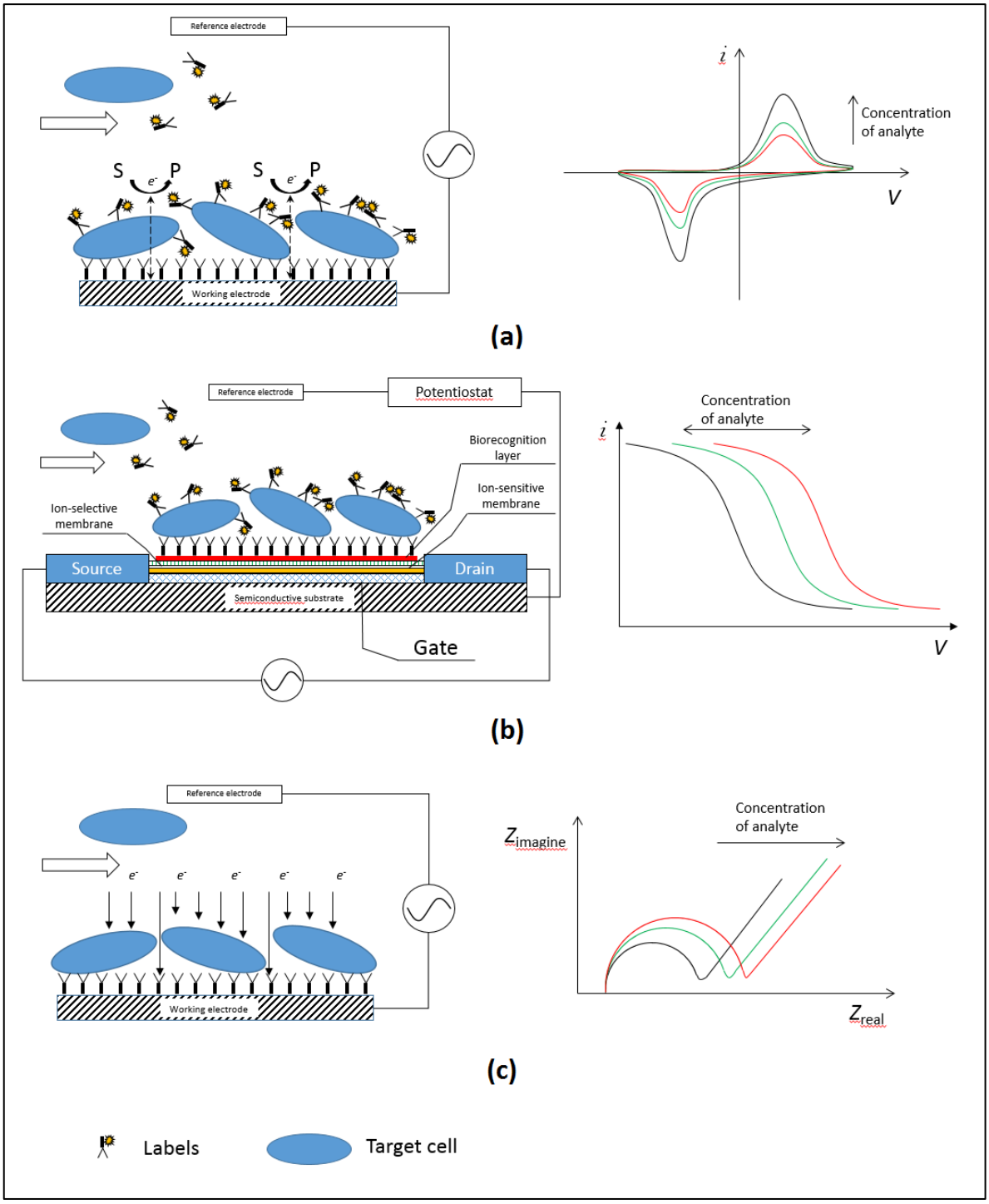


Figure 4

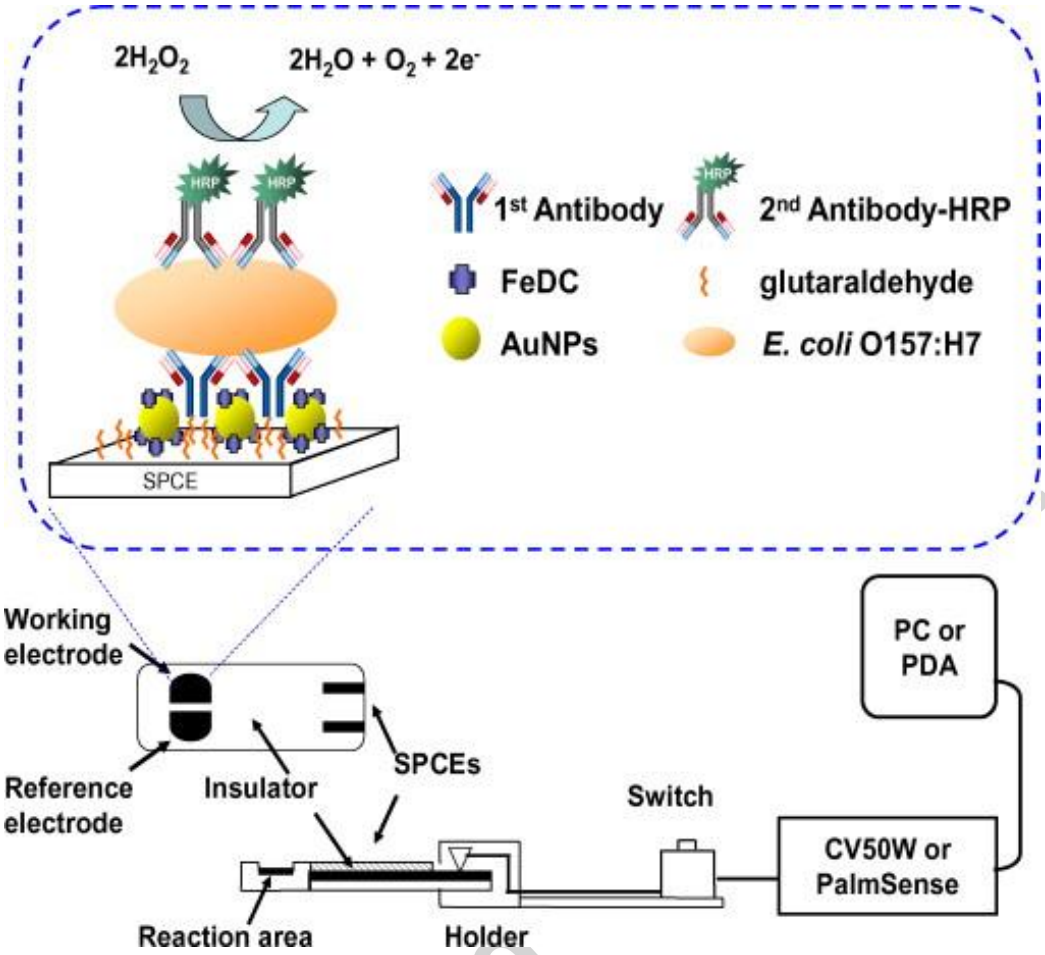
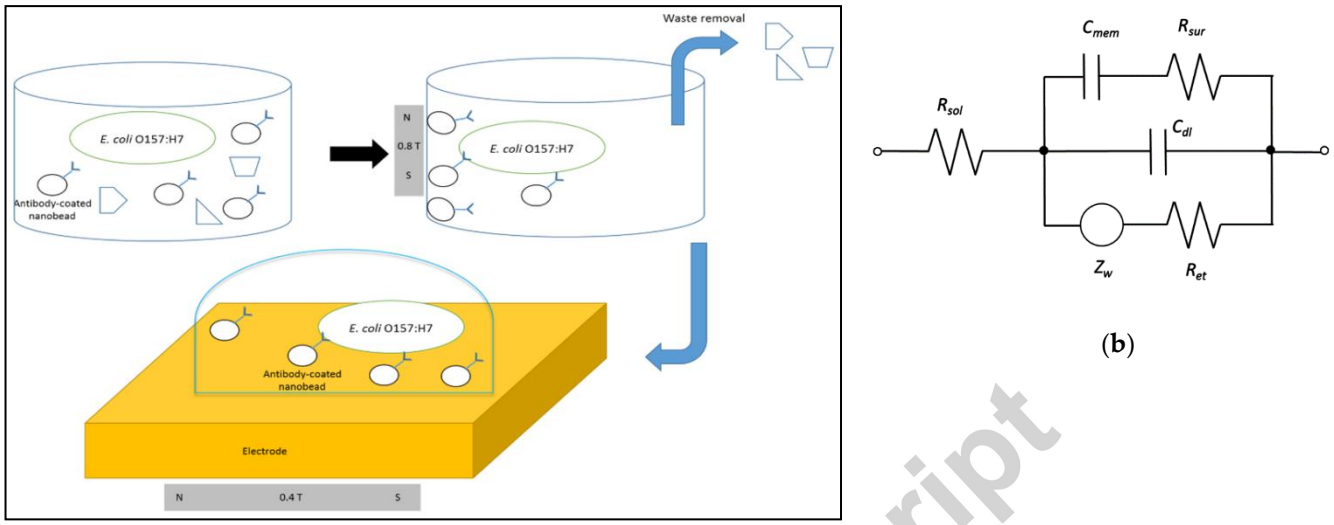
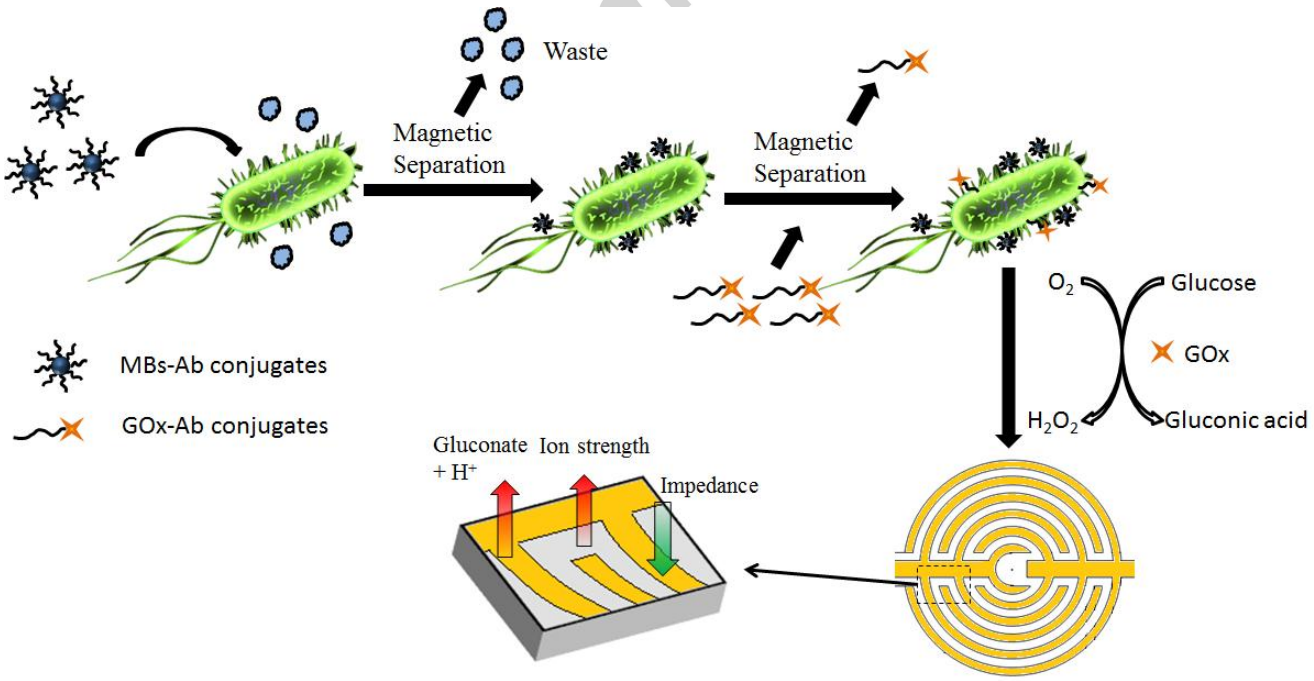


Figure 5


Waste removal
E. coli O157:H7
Antibody-coated nanobead
N
0.8 T
S
E. coli O157:H7
E. coli O157:H7
Antibody-coated nanobead
Electrode
N
0.4 T
S
C_{mem}
R_{sur}
R_{sol}
C_{dl}
Z_w
R_{et}

(**a**) (**b**)

Figure 6


Waste
Magnetic Separation
Magnetic Separation
MBs-Ab conjugates
GOx-Ab conjugates
O_2
Glucose
GOx
H_2O_2
Gluconic acid
Gluconate + H^+
Ion strength
Impedance

e 7

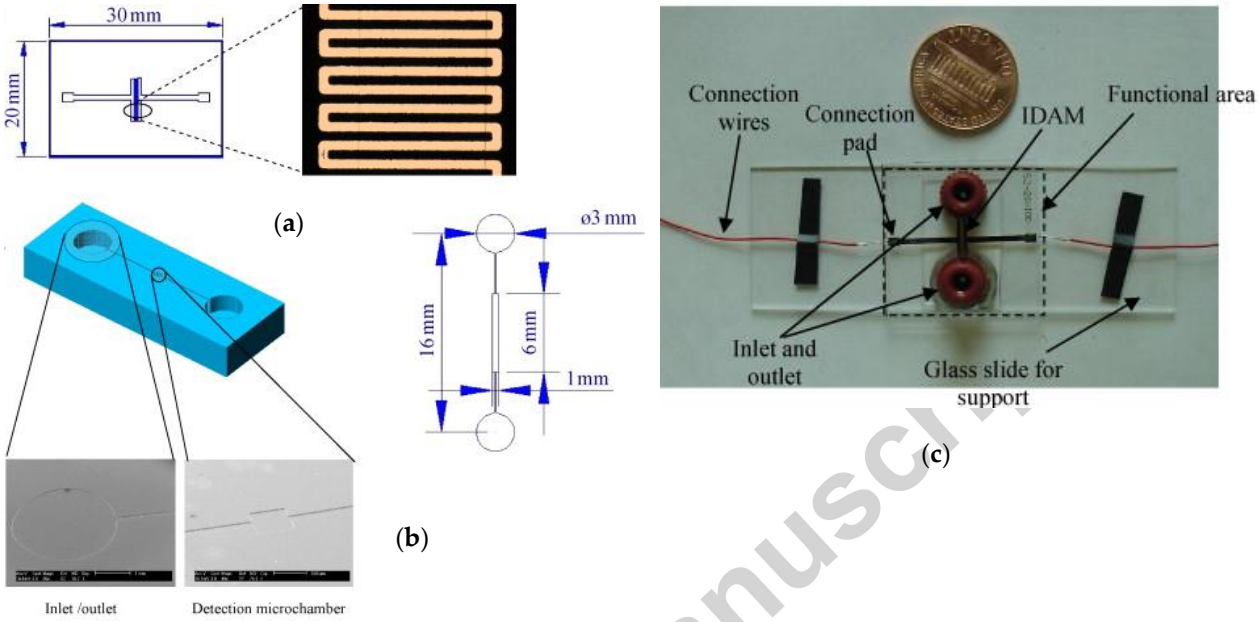


Table 1. Electrochemical biosensors for the detection of *E. coli* O157:H7 (unless particular stated).

Mode of transducers	Pathogen	Immobilization on electrodes	Labelling	LODs (cfu ml^{-1})	Assay time	Ref.
Amperometric/voltammetric		MBs + magnetized graphite electrode	Alkaline phosphatase	1.5×10^3 in apple juice	80 min	[30]
		SPCE/FeDC-AuNPs/ABs	HRP	5×10^3 in milk	30 min	[32]
	E. coli O157:H7, *Salmonella*, and *Campylobacter*	SPE/MWCNT-PAH/ABs	Respective ABs conjugated with CdS, CuS, or PbS nanocrystals	800, 400, and 400 in milk	> 1 h	[40]
		Bare SPCE	MBs-1st AB +AuNPs-	148 in buffer, 457 in	> 1 h	[41]

			2^{nd} AB	minced beef, and 309 in tap water		
		Au electrode/SAM of AUT/AuNPs/CHIT-MWNTs-SiO_2/THI/ABs	Label-free	250	~ 30 min	[48]
Impedimetric/ conductometric		Au electrode/SAM of neutravidin/biotin-ABs	Label-free	10 in PBS	~ 1 h	[43]
		Bare IDME, MBs-ABs-cell attracted by a magnet	Label-free	7.4×10^3 in buffer, 8.0×10^3 in ground beef	35 min	[44]
		quartz crystal Au electrode/protein A/ABs	Label-free	10^3 in buffer	10 min	[45]
	E. coli K12	Quartz substrate/graphene/ABs	Label-free	10 in buffer	30 min	[49]
		Working electrode//alumina nanoporous membrane/HA/ABs	Label-free	10 in buffer, 84 in whole milk	Not stated	[50]
		Au disk electrode/SAM of MHDA/ABs	Label-free	2 in buffer	45 min	[51]
		rGOP/AuNPs/ABs	Label-free	1.5×10^2 in buffer	30 min	[52]
		SPCE/graphene/AuNPs/ABs	Label-free	1.5×10^3 in buffer	Not stated	[53]
		ITO electrode/epoxysilane/ABs	Label-free	1 in buffer	45 min	[54]
Potentiometric	*E. coli* surrogate CECT 675	GCE/SWCNT/aptamers	Label-free	6 in milk, 26 in apple juice	Real-time	[46]

Capacitive	Quartz crystal Au electrodes/SAM of MPA/ABs	Label-free	10^2 in buffer, 10^3 in food samples	~ 1 h	[47]

Table 2. The desired properties of an ideal biosensor for foodborne pathogens detection.

Properties	**Value or quality**
Sensitivity	Less than 10^3 cfu ml^{-1}
Specificity	Can identify specific target strain from other serotypes in the same or different species (*e.g.*, can distinguish *E. coli* O157:H7 from other *E. coli* or *Salmonella*)
	Minimum background noise (minimum unspecific binding from the food matrices)
Detection time	5-10 min for single test
Size	Portable, compact instrumentation can fit into a suitcase
Consistency	The test can be performed at different conditions at the site of interest, and test results have no significant difference from those done at laboratory
Stability	The biorecognition elements or biochemical labels should be stable for months under normal condition for easy preservation
Sample processing	Minimal sample pre-treatment, simple test procedures (better to be label-free)
Operator requirement	No special training needed to use the assay, can be used by individuals at home

Highlights

- Comprehensive review on electrochemical biosensors to detect *E. coli* O157:H7 from 2000 to 2015.
- Label-based and label-free detection strategies are emphasized to classify the developed methods.
- New techniques that can improve the performance of electrochemical biosensors are discussed.
- Current challenges and future outlook are proposed.