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Rapid detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in foods using an electrochemical immunosensor based on screen-printed interdigitated microelectrode and immunomagnetic separation

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ABSTRACT

Foodborne pathogens have continuously been a serious food safety issue and there is a growing demand for a rapid and sensitive method to screen the pathogens for on-line or in-field applications. Therefore, an impedimetric immunosensor based on the use of magnetic beads (MBs) for separation and a screen-printed interdigitated microelectrode (SP-IDME) for measurement was studied for the rapid detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in foods. Streptavidin coated MBs were functionalized with corresponding biotinylated antibodies (Ab) to capture the target bacteria. The glucose oxidase (GOx)–Ab conjugates were employed to label the MBs–Ab–cell complexes. The yielded MBs–Ab–cell–Ab–GOx biomass was mixed with the glucose solution to trigger an enzymatic reaction which produced gluconic acid. This increased the ion strength of the solution, thus decreasing the impedance of the solution measured on the SP-IDME. Our results showed that the immunosensor was capable of specifically detecting *E. coli* O157:H7 and *S.* Typhimurium within the range of 10^2–10^6 cfu ml^{-1} in the pure culture samples. *E. coli* O157:H7 in ground beef and *S.* Typhimurium in chicken rinse water were also examined. The limits of detection (LODs) for the two bacteria in foods were 2.05×10^3 cfu g^{-1} and 1.04×10^3 cfu ml^{-1}, respectively. This immunosensor required only a bare electrode to measure the impedance changes, and no surficial modification on the electrode was needed. It was low-cost, reproducible, easy-to-operate, and easy-to-preserve. All these merits demonstrated this immunosensor has great potential for the rapid and on-site detection of pathogenic bacteria in foods.

1. Introduction

Over decades, the illnesses caused by foodborne pathogenic bacteria have had an enormous impact on public health and the economy [1]. In the United States, the Centers for Disease Control and Prevention (CDC) [2] has estimated that 31 types of known foodborne pathogens cause around 9.4 million illnesses, 56,000 hospitalizations and 1300 deaths annually. Among these 31 well-known pathogens, *E. coli* O157 and *Salmonella* are two major types that have been studied the most as models to understand the bacterial behavior. Latest outbreaks of *E. coli* O157:H7 in ground beef and *S.* Typhimurium in poultry have caused serious impact on the health of the public and the economy of food companies [3,4]. As the presence of foodborne pathogens is a major concern for the food industry, the detection of these pathogens has become utmost important. Conventional methods such as culturing and colony counting, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are still the most common methods applied in the field of pathogen detection [5]. In despite of their good selectivity and reliability, these conventional methods have disadvantages. For instance, the culturing and colony counting method is usually time-consuming and laborious due to multiple steps of enrichment and growth of the bacteria [6]. ELISA in 96-well microtiter plates is restricted by its relatively high LODs (normally 10^3–10^5 cfu ml^{-1}). In order to achieve low LODs, the ELISA method requires certain enrichment procedures that take 16–24 h, and is considered a long and labor-intensive analysis [7,8]. PCR-based methods, considered as rapid detection methods, can achieve detection results from 30 min to a few hours. However, the development of PCR-based rapid methods is still hindered by the requirement of enrichment, high cost, and the need

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for trained technicians [5].

The impedimetric biosensing technique, one branch of electrochemical methods, has proven to be very promising in the development of rapid methods for the detection of foodborne pathogens, especially for on-site detection. This technique applies a sinusoidal electrical signal with very low voltage in a set range of frequencies on an electrochemical cell [9]. The capture of bacterial cells onto the surface of the electrodes or fluctuations of ion strength in medium induced by the addition of chemical constituents would change the conductance and the capacitance of the medium thereby changing its impedance [10]. The predominant strategy for an impedimetric biosensor is the immobilization of bioreceptors (e.g., antibodies) onto the surface of the electrode. This strategy bypasses the labeling procedure that is normally required by other electrochemical biosensors, and greatly shortens the detection time. The charge transfer resistance increases when target bacteria are directly bound to the surface of the electrode [11–13]. However, despite the advantages brought by its label-free nature, the major drawbacks of impedimetric biosensors using the immobilization strategy include: (1) the immobilization procedures are complicated and time-consuming, (2) the consistency of the fabricated biosensors is greatly affected by the surface condition of the electrode and the unspecific absorption of compounds in biological samples, and (3) it is very difficult to reproduce and regenerate the electrode [14]. All of these disadvantages have restricted the development and application of impedimetric biosensors using the immobilization strategy in the field of food safety and inspection. Therefore, an immobilization-free detection method that can sensitively detect the pathogenic bacteria in foods would be very promising for the application of biosensing in the food industry.

In this study, an immobilization-free electrochemical impedance immunosensor was developed for the detection of pathogenic bacteria, *E. coli* O157:H7 and *S.* Typhimurium, in foods. The SP-IDME for measurement in this immunosensor was only used for the detection of *E. coli* O157:H7 [15] and avian influenza [16] previously in our lab. However, the first study was done using self-assembled monolayers (SAMs) of 3-dithiobis-(sulfosuccinimidylpropionate) (DTSP) to modify the surface of the SP-IDME, whereas the latter one targeted viruses using bare SP-IDME for measurement. Considering to the low-cost and reusability showed with the bare SP-IDME, the applications of this electrode in the detection of pathogenic bacteria are worth to explore. GOx, due to its high turnover number, high specificity, and good stability (pH 2–8), has been chosen as the labeling enzyme [17]. For the fabrication of the immunosensor, the magnetic beads (MBs) (130 nm) coated with streptavidin were first functionalized with the appropriate biotinylated Ab. *E. coli* O157:H7 or *S.* Typhimurium was then captured by the MBs–Ab conjugates. Once the target bacteria were captured, corresponding Ab–GOx conjugates were used to label the bacteria by forming MBs–Ab–cell–Ab–GOx sandwich biomass. The final biomass was transferred to the glucose solution with low ion strength. Through the enzymatic reaction, the ion strength of the aqueous samples increased whereas the impedance of the samples decreased. The impedance spectra were obtained using electrochemical impedance spectroscopy (EIS). The immunosensor was able to detect the target bacteria at 10^2 cfu ml^{-1} and differentiate them from non-target bacteria in the pure cultural samples. This study demonstrated that the proposed method using a bare SP-IDME could achieve similar sensitivity to those methods using immobilization strategies. Furthermore, this method is easy-to-operate and the electrode can be regenerated multiple times for long-term use. It is very suitable for the demand of on-site detection of pathogenic bacteria in foods.

2. Materials and methods

2.1. Biochemical materials

Stock phosphate buffered saline (PBS, 0.1 mol l^{-1}, pH 7.4), glucose, glucose oxidase, and bovine serum albumin (BSA) were bought from Sigma-Aldrich (St. Louis, MI). Stock PBS solution was diluted at a ratio of 1:10 to prepare 1x PBS (10 mmol l^{-1}, pH 7.4), and used throughout all tests. 1% BSA solution (wt/vol) was prepared in PBS as a blocking buffer. The ultrapure deionized water (18.2 MΩ cm) was obtained from Millipore (Milli-Q, Bedford, MA). 10 mmol l^{-1} glucose solution was made by dissolving glucose into deionized water. Streptavidin-coated magnetic beads (MBs) with a diameter of 130 nm were manufactured by Kisker Biotech GmbH&Co. KG (Steinfurt, Germany). Based on the information provided by the company, the MBs were prepared by precipitation of iron oxide (Fe_3O_4) in the presence of dextran, and contained 10 mg ml^{-1} solid content (Fe) with 2.9×10^{11} particles mg^{-1}. The surface of MBs was covalently (carbodiimide method) modified with 1.5 μg mg^{-1} particles (approximately 60–70 molecules streptavidin per particle). SEM and TEM images were taken to confirm the size of MBs, and multiple binding of MBs to bacterial cells (Fig. S1).

Biotinylated rabbit anti-*E. coli* O+K antibodies (4.0–5.0 mg ml^{-1}) were purchased from Biodesign International (Saco, ME). Monoclonal anti-*S.* Typhimurium antibodies (4.0–5.0 mg ml^{-1}) were purchased from Meridian Life Science Inc. (Memphis, TN). The anti-*S.* Typhimurium antibodies were biotinylated based on the protocol reported by Kanayeva et al. [18] using sulfo-NHS-biotin, and excessive biotin was removed with a Slide-A-Lyzer dialysis kit from Pierce Protein Research Product (10K MWCO, Rockford, IL). 1:5 dilutions of both antibodies (0.8–1.0 mg ml^{-1}) were prepared with PBS and stored at 4 °C for further use, and storage time was no more than one month.

Stock bacterial cultures of *E. coli* O157:H7 (ATCC 43888), *E. coli* K12 (ATCC 29425), *S.* Typhimurium (ATCC 14028), *Listeria monocytogenes* (ATCC 43251), and *Staphylococcus aureus* (ATCC 27660) were obtained from American Type Culture Collection (ATCC, Manassas, VA). The stock cultures were stored at −80 °C, and revived slowly at room temperature when needed.

2.2. Apparatus and electrodes

The impedance analysis was conducted with a bench-top IM-6 Impedance Analyzer manufactured by BAS-ZAHNER (West Lafayette, IN) with the software IM-6/THALES. For all impedance measurements, a sinusoidal AC module with amplitude of 5 mV was applied across the IDME, and the magnitude and the phase angle of the impedance were measured in the frequency range from 10 Hz to 1 MHz. The SP-IDME was designed by our group and fabricated by Aibit Tech. (Jiangyin, China). The configuration of the SP-IDME and the electric connection is shown in Fig. 1.

The bare electrode is constructed with six pairs of gold fingers arranged in a pattern of interdigitated concentric circle. The gold fingers are 200 μm in width with 200 μm spacing between each two fingers. The area of the gold fingers is about 12.38 mm^2, and the ratio of the area of the gold fingers to the whole circular electrode is about 0.54:1. The magnetic separator with a magnetic strength of approximately 1.0 T (Tesla) was purchased from R&D Systems Inc. (Minneapolis, MN).

2.3. Culture preparation and media plating methods

For preparing the test culture, the stock cultures were grown in brain heart infusion (BHI) broth (Remel Microbiology Products, Lenexa, KS) at 37 °C for 18–20 h. A series of 10-fold dilutions of