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An optical biosensor using immunomagnetic separation, urease catalysis and pH indication for rapid and sensitive detection of *Listeria monocytogenes*

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ABSTRACT

Early screening of pathogenic bacteria in foods plays an important role in foodborne disease prevention and control. In this study, we developed an optical biosensor for rapid and sensitive detection of *Listeria monocytogenes* combining immunomagnetic separation, urease catalysis and pH indication. The magnetic nanobeads (MNBs) conjugated with monoclonal antibodies (MAbs) by streptavidin-biotin binding were used for specifically and efficiently separating the *Listeria* cells from background to form magnetically labeled bacteria. Then, the gold nanoparticles (AuNPs) modified with urease and polyclonal antibodies (PAbs) by electrostatic adsorption were used to react with the magnetically labeled bacteria to form the MNB-MAb-*Listeria*-PAb-AuNP-urease complexes. The urease in the complexes could catalyze the hydrolysis of the urea into ammonium carbonate, which could lead to an increase in the pH value of the urea solution. Bromcresol purple was compared with phenol red and bromothymol blue and selected as the best pH indicator to monitor the color change, which could be measured at the characteristic wavelength of 588 nm for bacteria quantification. This proposed biosensor was able to detect *Listeria monocytogenes* as low as 1.0×10^2 CFU/mL, and had the mean recovery of 95.1% for *Listeria* in the spiked lettuce samples. This optical biosensor has showed its potential to provide a simple, low-cost and sensitive detection method for prevention and control of foodborne diseases.

1. Introduction

It was reported by WHO that diarrhoeal diseases caused by eating norovirus or foodborne pathogens contaminated foods led to 550 million illnesses and 230,000 deaths every year, and children were at high risk [1,2]. According the statistics from the National Health and Family Planning Commission of China, foodborne pathogens were responsible for over 50% of foodborne illnesses in the past decade [3]. The sensitive and rapid detection of foodborne pathogens is crucial for preventing and controlling the outbreaks of foodborne diseases.

Currently, the available strategies for the detection of foodborne pathogens mainly include traditional culture plating (gold standard method) [4], nucleic acid amplification-based methods (qPCR and mPCR) [5,6], immunological reaction-based methods (ELISA and Strip) [7,8]. However, these methods usually have the limitations of either long detection time (Culture), or sophisticated nucleic acid extraction (PCR), or lack sensitivity and specificity (ELISA and Strip). Therefore, novel strategies with shorter detection time, higher sensitivity and simpler operation are urgently needed for food safety.

As an alternative, various biosensors, such as optical biosensors, electrochemical biosensors and QCM biosensors, etc., have attracted increasing attentions due to simpler operation, online monitoring, shorter detection time and higher sensitivity in the detection of foodborne pathogens [9]. Comparing to other biosensors, optical biosensors, which generally rely on light absorbance, fluorescence, luminescence, reflectance, Raman scattering or refractive index, are more attractive due to rapidness, non-contact detection and compact design [10]. The strategy of catalyzing a substrate to produce a catalysate in a different color is one of the mainstreams of the color-dependent optical biosensors. The horse radish peroxidase (HRP, E.C.1.11.1.7) and alkaline phosphatase (AP, E.C.3.1.3.1) are the most commonly used enzyme labels, however they are present in many mammalian tissues and this greatly limits their applications in the assays with the whole cells as substrate. In addition, the commonly used substrate, hydro-

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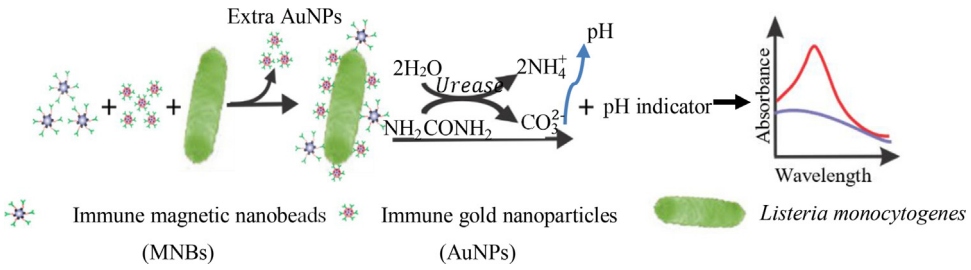

Fig. 1. Schematic of the optical biosensor based on immunomagnetic separation, urease catalysis and pH indication.

gen peroxide, is unstable [11,12] and this has great impact on the repeatability. Thus, many enzymes that can catalyze their substrates to change their pH values, such as urease [13,14], glucose oxidase [15,16], esterase [17], penicillinase [18], organophosphate paraoxon [19] were reported. Among them, urease receives the most attentions due to its high catalytic efficiency, low cost, and non-toxic substrate and catalytic product. So far, the common pH change based biosensors are electrochemical [20–22,16], and they often require expensive instruments, complex data processing, strong anti-interference capability and/or direct contact measurement.

Therefore, in this study we developed a novel optical biosensor with immunomagnetic separation, urease catalysis and pH indication for rapid and sensitive detection of foodborne pathogens. As shown in Fig. 1, the *Listeria monocytogenes* cells were first isolated and concentrated by magnetic nanobeads (MNBs) modified with the anti-*Listeria* monoclonal antibodies (MAbs) to form the *Listeria*-MNB complexes (magnetically labeled bacteria), and then incubated with gold nanoparticles (AuNPs) modified with the anti-*Listeria* polyclonal antibodies (PAbs) and the urease to form the urease-AuNP-*Listeria*-MNB complexes (enzymatically labeled bacteria). After successive washing with sodium chloride solution and deionized water to remove the redundant AuNPs, the urea prepared by deionized water was used to resuspend the complexes and hydrolyzed under the catalysis of the urease on the complexes into ammonium carbonate [23,24], resulting in an increase on the pH values of the urea solution. Finally, the urea solution was magnetically separated and mixed with the optimal pH indicator, bromocresol purple, and the color change was measured by the spectrometer for quantitative detection of the *Listeria* cells.

2. Materials and methods

2.1. Materials and reagents

The carboxylated magnetic nanobeads (180 nm, Fe content: 10 mg/mL) were purchased from Allrun Nano (PM3-020, Shanghai, China) and modified with the monoclonal antibodies by EDC method for immunomagnetic separation of the *Listeria* cells. The anti-*Listeria* monoclonal and polyclonal antibodies were provided from Zodolabs Biotech (Nanchang, China) for specifically binding with the *Listeria* cells. Gold chloride tri-hydrate ($HAuCl_4{\cdot}3H_2O$) and sodium citrate purchased from Sigma Aldrich (St. Louis, MO, US) were used to synthesize the gold nanoparticles (~20 nm). Streptavidin, long-chain biotin, urease (E.C.3.5.1.5, Type III, 15,000–50,000 units/g solid), urea (NH_2CONH_2), ammonium carbonate ($(NH_4)_2CO_3$), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 2-(N-morpholino) ethanesulfonic acid (MES), and phosphate buffered saline solution (PBS, P5493, 10 mM, pH 7.4) were also purchased from Sigma Aldrich. Bovine serum albumin (BSA) from EM Science (Gibbstown, NJ, US) and polyethylene glycol (PEG) 20,000 from Merck (Darmstadt, Germany) were prepared in PBS for surface blocking to avoid non-specific adsorption. Tween-20 was purchased from Amresco (Solon, OH, US) for washing. Bromocresol purple, bromothymol blue and phenol red were purchased from Sinopharm Chemical (Shanghai, China) as the pH indicators. Ultra-micro cuvettes (volume: 70–850 μL) were purchased from BrandTech Scientific (759200, CT, USA). The spectrometer from Ocean Optics (USB 4000, Dunedin, FL, USA) was used for optical measurement. Other reagents were of analytical grade and purchased from Sinopharm Chemical (Shanghai, China). All the solutions were prepared with deionized water produced by Advantage A10 from Millipore (Billerica, MA, USA).

2.2. Selection and optimization of the pH indicator

The pH indicator is an important material for this proposed optical biosensor. Three pH indicators, including bromocresol purple, bromothymol blue and phenol red, were selected and compared for the determination of the pH change of the urea solution after the catalysis of the urease. 2 mg of these pH indicators were dissolved in 8 mL of the deionized water containing 20% absolute ethyl alcohol as stock solution, respectively, and were stored at 4 °C. Prior to test, 20 μL of each pH indicator at the concentration of 1/40 mg/mL were placed in the same row (see Fig. 2). 200 μL of ammonium carbonate with different concentrations ranging from 1 μM to 100 μM at the presence of 100 μM of urea was used to simulate the catalyzed ammonium ions and carbonate ions and react with the pH indicators for 1 min. The color changes were measured using the USB 4000 spectrometer with the ultra-micro cuvettes at the wavelength range from 400 nm to 800 nm.

After the best pH indicator was selected, its stock solution at the original concentration of 1/4 mg/mL was first diluted with the deionized water to obtain the pH indicator at the concentrations of 1/40 mg/mL, 1/80 mg/mL and 1/160 mg/mL for the optimization on the concentration of the indicator. Then, 20 μL of the pH indicators at each concentration were placed in the centrifuge tube, respectively, and mixed with 200 μL of ammonium carbonate with different concentrations, followed by incubation for 1 min, and the mixtures were immediately transferred into the ultra-micro cuvette for absorbance measurement using the spectrometer.

2.3. Culture and enumeration of the bacterial cells

Listeria monocytogenes (ATCC 13932, used as target bacteria) and *Escherichia coli* O157:H7 (ATCC 43888, used as non-target bacteria) were purchased from the American Type Culture Collection, and were revived by streaking on Luria-Bertani (LB) agar plates. They were first inoculated into the LB medium (Aoboxing Biotech, Beijing, China) for 12–16 h at 37 °C with shaking at 180 rpm, respectively. Then, the bacterial cultures were serially 10-fold diluted with PBST (PBS with 0.05% Tween-20) to obtain the bacteria samples at the concentrations from 10^2 to 10^6 CFU/mL, respectively.

For the enumeration of the viable bacterial cells, 100 μL of each dilution of the samples was surface plated onto the LB agar plate,