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Portable and quantitative point-of-care monitoring of *Escherichia coli* O157:H7 using a personal glucose meter based on immunochromatographic assay

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ABSTRACT

Here we innovate a portable and quantitative immunochromatographic assay (ICA) with a personal glucose meter (PGM) as readout for the detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7). The carboxyl group coated Fe_3O_4 nanoparticles (MNPs) were synthesized by a one pot method and used as carriers of invertase and monoclonal antibody against *E. coli* O157:H7. Initially, the invertase and antibody double functionalized MNPs (Invertase-MNPs-IgG) conjugates were prepared and used as label probe in this assay system. Before laminating onto the baking card, the absorbent pad was soaked in sucrose solution and desiccated. MNPs produced brown band at the detection zone of the ICA when acting as direct labels. As they were also coupled with invertase, the invertase catalyzed the hydrolysis of sucrose on the absorbent pad into glucose, which was detected by the PGM. To increase the sensitivity, antibody functionalized MNPs were used to enrich *E. coli* O157:H7 from sample solution. The innovative aspect of this approach lies in the visualization and quantification of *E. coli* O157:H7 through Invertase-MNPs-IgG and the detection of glucose concentration using PGM. Although the feasibility is demonstrated using *E. coli* O157:H7 as a model analyte, this approach can be easily developed to be a universal analysis system and applied to detection of a wide variety of foodborne pathogens and protein biomarkers. This study proposed a qualitative and quantitative analysis device for the clinic diagnostics and food safety analysis.

1. Introduction

Escherichia coli O157:H7 (*E. coli* O157:H7) is an important serotype of enterohemorrhagic *Escherichia coli* (*E. coli*) and can cause severe serious diseases in humans. *E. coli* O157:H7 can contaminate food and water, resulting in about 73000 illnesses occurring each year (Mead et al., 1999). At present, the common methods for detecting *E. coli* O157:H7 include culture method, the polymerase chain reaction (PCR) (Bonetta et al., 2016; Wang et al., 2017) and enzyme-linked immunosorbent assay (ELISA) (Amani et al., 2015), most of these methods require expensive instruments and specialized staff.

Immunochromatographic assay (ICA) is user-friendly and a point-of-care testing (POCT), which requires little training and can be applied in laboratories without precision equipment (Liu et al., 2009; Gao et al., 2014). ICA has been widely applied for POCT of toxic or harmful substances in food safety monitoring and on-site diagnosis (Fang et al., 2015). However, one drawback of conventional gold nanoparticles-based ICA is that it can only realize qualitative or semi quantitative analysis of target analytes, such assays can only be used for analyzing target analytes with relatively high concentrations. To realize quantitative detection of target analytes, fluorogenic or chemiluminescent substrates are used instead of gold nanoparticles. Unfortunately, the readout for fluorescence or chemiluminescence requires expensive instrumentation that increases the overall cost of the assay.

The personal glucose meter (PGM) is the most widely used point-of-care device in the world because of its reliable quantitative result, portable size, low cost and simple operation. By establishing a relationship between target recognition and glucose generation, PGM has been widely used to quantitatively detect non-glucose target. To broaden the applicability of PGMs, Lu and others (Xiang and Lu, 2011) have conjugated the aptamer or antibodies with an enzyme such as invertase or amylase, which can catalyze the conversion of non-glucose analysts into glucose. Zhang et al. (2016) first integrated solution-based assays onto lateral flow device and used the proposed lateral flow device-PGM system for quantitative detection of both cocaine and streptavidin. DNA-Invertase conjugates functionalized magnetic beads were spotted on the sample pad, and sucrose was applied on the reaction pad. The target molecular transferred the invertase from the sample pad to reaction pad where the sucrose was hydrolyzed to glucose (Zhang et al.,

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2016). Zhu et al. (2017) used a glucose meter and ICA strip to quantitative detection of 8-hydroxy-2-deoxyguanosine. Gold nanoparticles labeled antibody and 8-hydroxyguanosine–invertase conjugates were used to recognize target and establish the relationship between target recognition and glucose generation, respectively (Zhu et al., 2017). Based on these excellent works, we innovatived a qualitative and quantitative POCT of *E. coli* O157:H7 based on the ICA by using PGM as readout.

In the traditional ICA, colloidal gold has widely used as the marker material and color constituent. However, colloidal gold is easily affected by pH value and salt ions strength. And during the functionalization process, high speed and longtime centrifugation is necessary. Magnetic nanoparticles can be used to overcome such disadvantages. Zhao et al. has reported a facile one pot method for synthesis of highly water dispersible carboxyl group coated Fe_3O_4 nanoparticles (MNPs) (Jia et al., 2009). Here we developed a new method for one-step modifying MNPs with enzyme and antibody, and it does not require surface functionalization (Chen et al., 2015; Si et al., 2015; Yang et al., 2015).

The aim of this study is to set up a qualitative and quantitative analysis device for the clinic diagnostics and food safety analysis. Here, a PGM-based immunochromatographic assay (PGM-ICA) system was developed for portable and quantitative detection of *E. coli* O157:H7. The basic design of the ICA is a MNPs-based sandwich immunoassay. The MNPs were not only used as labels but also as carriers of enzymatic labels. The invertase and antibody against *E. coli* O157:H7 double functionalized MNPs (IMIc) were optimized as signal probe, which transformed the detection of the target to the detection of glucose.

2. Experimental

2.1. Materials and reagents

The *E. coli* O157:H7 (NCTC 12900), the mouse anti-*E. coli* O157:H7 monoclonal antibody for labeling (antibody A) and capture (antibody B) were purchased from Shanghai Prajna Biology Co. Ltd. (Shanghai, China). *Escherichia coli* (*E. coli*, ATCC 8739), *Staphylococcus aureus* (*S. aureus*, ATCC 29213), *Citrobacter freundii* (*C. freundii*, ATCC 43864), *Cronobacter sakazakii* (*C. sakazakii*, ATCC 29544), *Listeria monocytogenes* (*L. monocytogenes*, ATCC 19115), *Shewanella putrefaciens* (*S. putrefaciens*, ATCC 8071), *Bacillus subtilis* (*B. subtilis*, ATCC 6633), *Bacillus cereus* (*B. cereus*, ATCC 10987) and *Vibrio parahaemolyticus* (*V. parahaemolyticus*, ATCC 17802) were purchased from China Center of Industrial Culture Collection (Beijing, China), and conserved in our laboratory. 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxy-sulfo-succinimide sodium salt (sulfo-NHS), 2-[N-morpholino] ethane sulfonic acid (MES), polyoxyethylene sorbitan monolaurate (Tween-20), sucrose and trehalose were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Invertase (300 U/mg) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Nutrient broth culture medium, nutrient agar culture medium, *E. coli* O157:H7 chromogenic medium (PBE007), modified EC novobiocin broth culture medium (mEC + n) and novobiocin were purchased from Beijing Land Bridge Technology Co. Ltd. (Beijing, China). BCA Protein Assay Kit was from Thermo Fisher Scientific (Shanghai, China) Co. Ltd. The nitrocellulose (NC) membrane was from Sartorius. Conjugate pad was from Ahlstrom. Absorbent pad, baking card, antibody manual coated platform were provided by Joey-Bio (Shanghai, China).

2.2. Preparation of invertase-MNPs-IgG conjugates (IMIc) and MNPs-IgG conjugates (MIc)

2.2.1. Preparation of invertase-MNPs-IgG conjugates (IMIc)

Here we used a one-pot method for preparing MNPs according to the method reported by Zhao et al. with a slightly modification (Jia et al., 2009). Typically, 0.68 mmoL of trisodium citrate, 1.2 g of natrium aceticum and 4.0 mmoL of $FeCl_3{\cdot}6H_2O$ were dissolved in 20 mL of ethylene glycol under heating and stirring. The obtained uniform yellow solution was transferred to a Teflon-lined stainless-steel autoclave, sealed and heated at 200 °C for 10 h. The obtained magnetic nanoparticles were washed with ethanol and deionized water for several times, and then dried at 80 °C under vacuum.

In order to simplify the preparation process of double functionalized MNPs and to realize one-step modifying MNPs with proteins. BSA was replaced by anti-*E. coli* O157:H7 antibody A to block non-specific adsorption sites on MNPs. Briefly, 2 mg of MNPs was dispersed in 500 μL activation buffer (MEST, 10 mM, 0.05% Tween-20, pH 5.0), followed by the addition of EDC (2.5 mg) and NHS (2.5 mg), and stirred for 30 min. The products were washed and dispersed in 500 μL of MES buffer (10 mM, pH 4.2). MNPs surface was first incubated with 100 μg of invertase under stirring at 4 °C for 1 h, and then transfer to borate buffer (BB, 20 mM, pH 7.2), 20 μg of anti-*E. coli* O157:H7 antibody A was added and stirred for 3 h at 4 °C. The obtained IMIc were washed and redispersed in 100 μL of storage solution (BBT, 5 mM, 3% trehalose, 0.05% Tween-20, 0.1% BSA, pH 7.2) for further use.

2.2.2. Preparation of MNPs-IgG conjugates (MIc)

Firstly, 2 mg of MNPs were activated with EDC/NHS as mentioned above and washed by 500 μL of borate buffer two times. After dispersing in 500 μL of BB buffer (20 mM, pH 7.2), the activated MNPs was mixed with 30 μg of anti-*E. coli* O157:H7 antibody A, and stirring for 1 h at room temperature. And then, the mixture was incubated in 1% BSA for 1 h to decrease nonspecific binding. Next, the conjugates were washed by 500 μL of borate buffer repeatedly, and MIc were ready for separation and enrichment.

2.3. Preparation of the immunochromatographic test strip

The test strip was prepared as previously reported with some modifications (Zhang et al., 2016). Briefly, the three membrane pads used for assembling the immunochromatographic strip were as follows (from the bottom): a glass fiber membrane (4 mm × 20 mm) for sample application and the release of the IMIc, a nitrocellulose (NC) membrane (4 mm × 14 mm) for signal generation, and a cellulose membrane (4 mm × 26 mm) for sucrose absorption and continuous sample absorption. The monoclonal anti-*E. coli* O157:H7 antibody B (2 mg/mL) was sprayed onto NC membrane to form the test line (T) by antibody manual coated platform, and then dried at 37 °C for 2 h. The conjugate pad was blocked with 5 mM of BB (pH 7.2) solution containing 1% BSA, 0.1% Tween-20, 4% trehalose, 0.2% PEG 20,000, and absorbent pad was soaked in 0.5 M sucrose solution, followed by desiccating at 50 °C for 3 h. The conjugate pad, NC membrane and absorbent pad were orderly laminated onto the baking card by overlapping 2 mm among them. The whole assembled card was subsequently cut into 4 mm wide strips. Then the strips were stored in a self-sealing plastic bag at room temperature until use.

2.4. Bacterial strains and culture conditions

E. coli O157:H7 and non-*E. coli* O157:H7 were cultured at 37 °C for 18 h. And their cultures were detected by PGM-ICA directly. To count the number of viable cells, serial decimal dilutions of cultures in physiological saline were plated onto nutrient agar medium. The plates were subsequently incubated at 37 °C for 24 h till enumeration.

2.5. MIc enrichment and capture of E. coli O157:H7

The enrichment of *E. coli* O157:H7 was performed according to a previous report (Xiong et al., 2014). Typically, *E. coli* O157:H7 cultures were serially diluted to 10^5 cfu/mL in physiological saline. And then, different amount of MIc dispersions were mixed with 1 mL of *E. coli*