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Immunoassay for pathogenic bacteria using platinum nanoparticles and a hand-held hydrogen detector as transducer. Application to the detection of *Escherichia coli* O157:H7

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Abstract

An innovative approach is presented for portable and sensitive detection of pathogenic bacteria. A novel synthetic hybrid nanocomposite encapsulating platinum nanoparticles, as a highly efficient catalyst, catalyzes the hydrolysis of the ammonia–borane complex to generate hydrogen gas. The nanocomposites are used as a label for immunoassays. A portable hand-held hydrogen detector combined with nanocomposite-induced signal conversion was applied for point-of-care testing of pathogenic bacteria. A hand-held hydrogen detector was used as the transducer. *Escherichia coli* O157:H7 (*E. coli* O157: H7), as detection target, formed a sandwich structure with magnetic beads and hybrid nanocomposites. Magnetic beads were used for separation of the sandwich structure, and hybrid nanocomposites as catalysts to catalyze the generation of hydrogen from ammonia−borane. The generated hydrogen was detected by a hydrogen detector using an electrochemical method. *E. coli* O157:H7 has a detection limit of 10 CFU·mL^{-1}. The immunosensor made the hand-held hydrogen detector a point-of-care meter to be used outdoors for the detection and quantification of targets beyond hydrogen.

Keywords Platinum nanoparticles · Hybrid nanocomposites · Immunosensor · Hydrogen gas · LOD · Point-of-care

Introduction

Point-of-care (POC) immunoassays are beneficial for the detection of pathogenic bacteria or monitoring biomarkers related to human health, and can be used at home or in the field. POC immunosensors are especially suitable for use in

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resource-limited environments because of their simplicity, accuracy, and low cost [1–3]. Although POC testing has grown rapidly over the past few decades [4], only a limited number of products, such as personal glucose meters (PGMs) [5] and pregnancy test strips [6], are commercially available to the public. The portable POC immunosensor has attracted interest from scientists and engineers because it produces real-time signal readouts, especially in low-resource settings [7].

The manufacture of POC devices is challenging. POC testing systems usually comprise a target recognition component, transducer, and signal processor. The transducer plays an important role in POC testing, and converts target molecular recognition events into measured signals. In the last few decades, some portable devices (e.g., PGMs, thermometers, pH meters, and pressure meters) have been developed for POC detection depending on the principle of completely different signals. Multiple new approaches based on converting non-glucose targets into glucose have been developed so that the most widely used PGM is simply used to quantify targets (e.g., platelet-derived growth factor-BB, metal ions, toxins, and pathogens) [8–10]. In traditional applications, the pH

meter is used for detecting the concentration of hydrogen ions. pH meters also have been successfully used for detection of targets, from pathogens [11], to aflatoxin B1 [12], human oncogenic protein [13], and telomerase activity [14]. Thermometers [15] and pressure meters [16] have been successfully used as the signal readers in bioassays. The use of portable devices that are widely applied in daily life opens a new door for signal transduction strategies. However, these devices for the detection platform also have some shortcomings. For example, the respective interference of endogenous glucose and pH value in PGM and pH meter readouts in clinical samples is inevitable [17]. Pressure reading is sensitive to temperature and elevation changes. Ambient temperature changes affect thermometer readouts.

Hydrogen (H_2), as a clean energy source without carbon-based emissions and other pollutants, is a globally accepted clean energy carrier. The requirement for H_2 is increasing and H_2 is widely used in daily life [18]. For the safety of H_2 application, H_2 detectors have been universally used, especially portable hand-held devices. The portable hand-held H_2 detector has many attractive features, such as ease of use, portability, and accurate, sensitive H_2 detection. A major challenge in using a portable H_2 detector to assay targets (e.g., pathogenic bacteria) other than H_2 is to find an approach that can connect the detection target to a change of H_2 so that one simply detects the target and measures its concentration using the detector [15]. The efficient transformation of target recognition into generating H_2 is an important step for sensitive detection in such bioassays. In all existing reactions to generate H_2, the decomposition of 19.6 wt% high H_2 capacity ammonia–borane (NH_3-BH_3, denoted as AB) has gained a lot of attention. Remarkably, generating H_2 gas also has a nontoxic advantage. In theory, under standard conditions, 1 mmol AB is decomposed to produce 67.2 mL H_2. If the reaction system is in a sealing device, production of H_2 gas can easily be detected by a H_2 detector.

A number of metal nanoparticle (MNP) catalysts have shown excellent catalytic activity and recycling performance in AB dehydrogenation [19]. Among them, platinum (Pt) is one of the most widely studied catalysts because it has remarkable activity for hydrolytic dehydrogenation of AB [20]. MNPs need to be coupled with substances, such as antibodies, that have strong affinity to the detection target, and then formed protein–MNP conjugates are used as efficient signal-transduction tags for bioassays. It is important to construct high-performance protein–MNP conjugates in the POC immunosensor. Until now, multiple methods synthesizing high-performance protein–MNP conjugates have been developed. Complex preparation processes often sacrifice the protein recognition function of the protein–MNP conjugates. A type of protein and phosphate (e.g., $Cu_3(PO_4)_2$, $CaHPO_4$) hybrid nanocomposites has attracted interest because of one-step coprecipitation preparation and large surface–volume ratio.

Hybrid nanocomposites have been developed as a matrix to immobilize biological molecules, whose activity and stability are correspondingly enhanced. We have observed that the concanavalin A-$Cu_3(PO_4)_2$ nanocomposites embedded a large number of hemin particles, which has outstanding peroxidase activity [21].

In the present study, we synthesized peptide–$Cu_3(PO_4)_2$ hybrid nanocomposites with embedded PtNPs (PPNs), encapsulating many Pt particles, and the PPNs were an ideal immunosensor label for hand-held H_2 detector signal readouts, by transforming pathogenic bacteria recognition events into H_2 signals. Our adaptation of a hand-held H_2 detector for pathogenic bacteria POC detection by combining hybrid nanocomposites consisted of two steps (Scheme 1): 1) the peptide–inorganic hybrid nanocomposites was captured by immunomagnetic bead separation; and 2) the hand-held H_2 detector was used as a signal readout. The process of synthesizing hybrid nanocomposites at room temperature mixed antimicrobial peptides (AMPs) and PtNPs into phosphate-buffered saline (PBS) solution containing Cu^{2+} ions. AMPs have received increasing interest as promising biorecognition molecules for bioassays since they can bind to the surface of bacterial cells with high affinity and specificity. Using AMPs as recognition molecules, several bacterial biosensor platforms have been developed because of their stability, simple acquisition, cost-effectiveness, and standard composite protocols. PPNs had the ability to dehydrogenate AB to generate H_2, and can selectively recognize target bacteria through binding of peptide to the bacterial surface. In addition, the uniform size and regular shape of the nanocomposites promoted their consistent attachment to each of the bacterial cells, leading to good linearity in the assay of pathogenic bacteria, namely *E. coli* O157:H7. Integration of hybrid nanocomposites–dehydrogenated AB and immunomagnetic separation led to a selective and sensitive gas signal method of detecting *E. coli* O157:H7.

To the best of our knowledge, this study is the first to develop an assay based on hand-held H_2 detector readouts and nanocomposites signal amplification for pathogenic bacteria detection. A sensitive and selective method for the detection of *E. coli* O157:H7 was developed. The limit of detection (LOD) of the POC immunosensor was 10 CFU·mL^{-1} in a linear range from 10 to 10^4 CFU·mL^{-1}.

Materials and methods

Reagents

PtNPs were provided by Sigma–Aldrich (St. Louis, MO, USA, www.sigmaaldrich.com/china-mainland.html). NH_3-BH_3 were obtained from Hwrk Chem Co. Ltd. (Beijing, China, www.hwrkchemical.com). The AMPs (Magainin I:
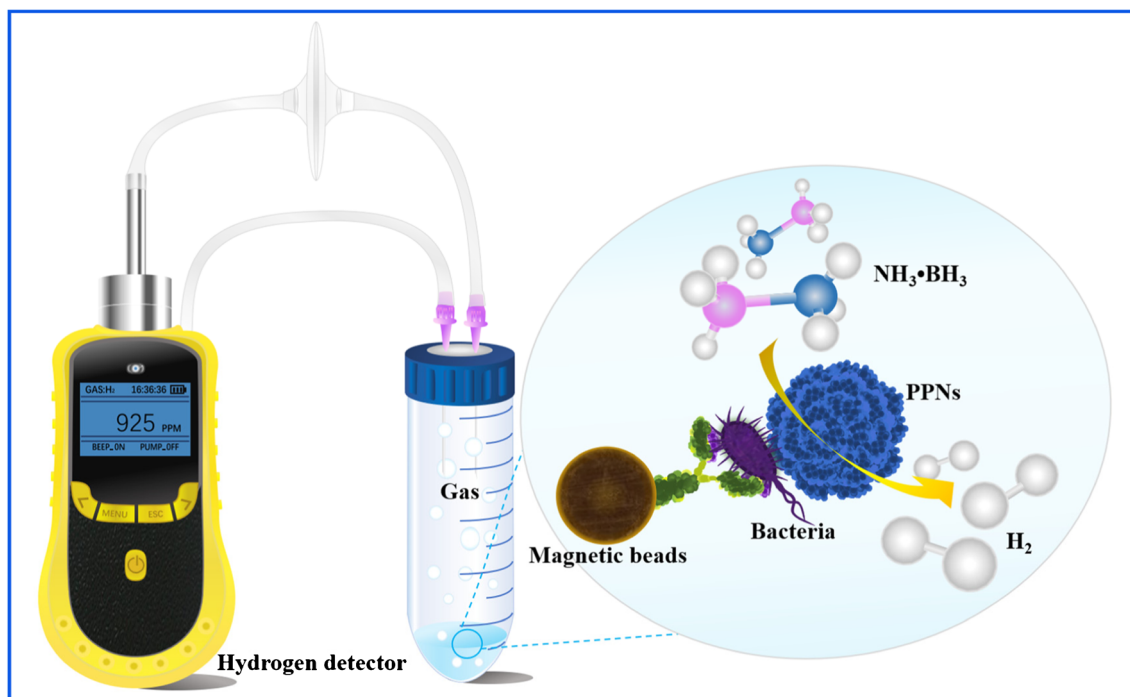
Scheme 1 Schematic representation of the immunosensor for pathogenic bacteria using a H_2 detector as signal readout

GIGKFLHSAGKFGKAFVGEIMKS) were designed as described previously [22] and obtained from Sangon Biotech Co. Ltd. (Shanghai, China, www.sangon.com). Magnetic beads (MBs) group-modified with streptavidin (average diameter 2.8 μm) were purchased from Invitrogen (Waltham, MA, USA, www.thermofisher.com). *E. coli* O157:H7 polyclonal antibodies coupled with biotin (AB20640) were obtained from Abcam (Hong Kong, China, www.abcam.cn). $CuSO_4 \cdot 5H_2O$ were purchased from Sangon Biotech (Shanghai, China, www.sangon.com). PBS consisted of 0.01 M NaH_2PO_4, 0.01 M Na_2HPO_4 and 0.014 M NaCl (pH 7.4). The hand-held hydrogen detector, which operated on principles of electrochemistry and thermal conductivity (SKY2000-H_2), was purchased from Unitec Technology (Shenzhen, China, www.szyuante.com/list-8.html). All chemicals were used without further purification. All aqueous solutions were made from ultrapure water (18. 3 MΩ· cm, Milli-Q; Millipore, Billerica, MA, USA, www.merckmillipore.com). Strains of *Salmonella* sp. (CMCC 50115), *Listeria monocytogenes* (Lis, BNCC 128913), *E. coli* O157:H7 (CICC 21530) and *Staphylococcus aureus* (BNCC 186335) were provided by the Academy of Military Medical Sciences (Beijing, China).

Synthesis of hybrid nanocomposites

PPNs with a diameter of about 4 μm were prepared according to the strand method with some modification [23]. Magainin I peptide aqueous solution (0.01 mg·mL^{-1}) and $CuSO_4 \cdot 5H_2O$ aqueous solution (20 μL of 120 mM) were added to 1 mL PBS (pH 7.4, 10 mM). PtNPs (0.9 mg·mL^{-1}) were introduced under mild stirring, and allowed to form the PPNs at room temperature for 24 h. The PPNs were obtained by centrifugation (8000 rpm), rinsed twice and dispersed in 1 mL 0.1 mM PBS solution (pH 6.0). The solution was stored at 4 °C for further use. The structure of the PPNs was determined by scanning electron microscopy (SEM; Hitachi SU8010, Tokyo, Japan).

Detection process of this assay

We designed an immunosensor for *E. coli* O157:H7, and the complete detection strategy is shown in Scheme 1. MBs labeled with antibody were synthesized according to protocol and used for separation and recognition of *E. coli* O157:H7 (via streptavidin–biotin interaction); mainly including 0.05 mg·mL^{-1} of *E. coli* O157:H7 polyclonal antibodies dissolved in 5 mg·mL^{-1} MBs. After 45 min incubation at 25 °C, the solution of MBs modified with *E. coli* O157:H7 antibody was washed three times with PBS to remove the unbound antibody. The antibody−MBs were finally kept at 4 °C until further use. MB-conjugated antibody was added to 10 μL of different concentrations of *E. coli* O157:H7, for recognition of the target *E. coli* O157:H7. The mixture was incubated at 35 °C for 80 min and magnetically separated by washing with PBS three times. The supernatants were removed to dispose of the excess nontarget bacteria. PPNs (4 μL) were added to the remaining MBs for 35 min at 35 °C. After addition of the
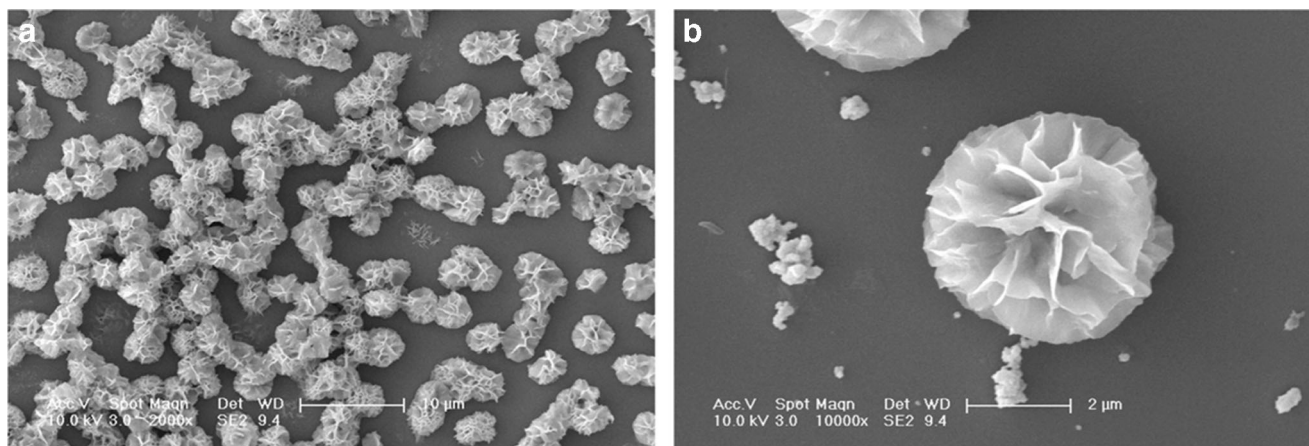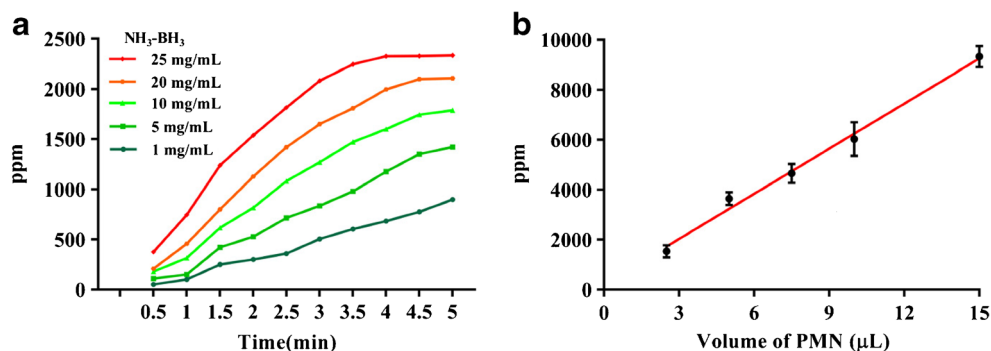
Fig. 1 SEM image of the PPNs at a scale of 10 μm (**a**) and 2 μm (**b**). The average size of the PPNs was about 4 μm

PPNs, the MBs–*E. coli* O157:H7 bound to the PPNs. The unbound PPNs were removed by magnetic separation. After another four washes with PBS, the mixture was resuspended in 500 μL PBS (0.1 mM, pH 6.0) and placed in a 50-mL centrifuge tube. As shown in Scheme 1, PPNs, after antibody-labeled MBs and the target of *E. coli* O157:H7, formed an immune−sandwich complex, and the signal output immunosensor can be acquired through this procedure. To exert the maximum catalytic effect of the PPNs, 10 μL NaOH (4 M) was added to destroy the nanocomposites to release the PtNPs. NH_3-BH_3 (1 mL, 25 mM) was injected through the pipe orifice of the centrifuge tube and the centrifuge tube was closed immediately with a rubber seal (double cover: the first layer was a common centrifugal tube cover with a circular hollow in the middle, and the second layer was a thin rubber layer). The needle of the digital H_2 detector was inserted into the rubber-sealed tube (the center of the centrifugal pipe was hollowed out to expose the rubber layer). The generation of H_2, which was utilized as a signal readout to detect target bacteria in the sealed centrifuge tube, was accurately measured by a portable H_2 detector and the digitally displayed value was read within 3 min. *E. coli* O157:H7 was quantitatively and qualitatively analyzed. Parts per million (ppm) was the unit of measurement of H_2 detection. P/P_0 represented the ratio of target *E. coli* O157:H7 in the presence and absence of 10^4 CFU·mL^{-1}.

Preparation of milk samples

For processing *E. coli* O157:H7 in real samples of milk, sterile milk was spiked by target pathogens. *E. coli* O157:H7 was diluted in PBS to a concentration of 10^2, 10^3, and 10^4 CFU·mL^{-1}. Milk was purchased from a local supermarket. The immune sandwich structure with the target bacteria was used as described above to calculate the recovery (%), on the calibration plot.

Statistical analysis

All data were analyzed using GraphPad Prism 5 and expressed as the mean ± standard deviation. All parameters were measured at least three times to validate repeatability and reproducibility.

Results and discussion

Synthesis and characterization of PPNs

The PPNs were observed using SEM (Fig. 1a, b). According to the SEM characterization (Fig. S1), the PPNs had an average diameter of about 4 μm. This was consistent with the previous traditional organic–inorganic hybrid nanocomposites structure [23, 24].

Fig. 2 **a** Time-dependent changes in pressure with different concentrations of AB (PPN = 2.5 μL) and (**b**) linear relationship between H_2 signal and volume of PPNs in the catalytic dissociation of AB (5 mg·mL^{-1}) for 5 min at room temperature

Fig. 3 a Sensitivity of the assay using a H_2 detector for detecting *E. coli* O157:H7 in the range of 10–10^7 CFU·mL^{-1}. **b** Linear relationship between the intensity of H_2 signal and log of *E. coli* O157:H7 concentration. Each value represents the mean of three independent experiments ($n = 3$)
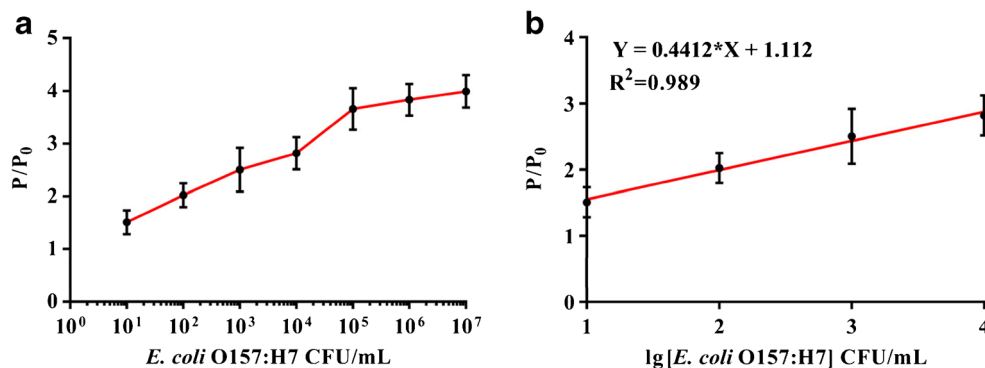
AB is a promising chemical H_2 storage material, which can be released by hydrolysis. The acquired PPNs were tested for catalytic hydrolysis of AB. The reaction was activated by addition of aqueous solution of AB into the reaction tube, comprising the PPN catalyst at room temperature. Figure 2a shows the time-dependent changes in H_2 detector and the concentration of H_2 produced as a function of target bacterium concentration. This change was directly converted to the H_2 detector readout. The linear relation between the volume of PPNs in AB and the final concentration of H_2 was apparent (Fig. 2b). This catalytic dissociation activity demonstrated that the synthesized PPNs had excellent AB decomposition efficiency and can be successfully used in H_2 detector assays.

Optimization of conditions

Different conditions were investigated: (a) number of PtNPs; (b) concentration of Magainin I; (c) reaction temperature; (d) reaction incubation time; (e) pH value; and (f) concentration of NaOH.

The following experimental conditions were able to achieve the best results: (a) umber of PtNPs, 0.9 mg·mL^{-1}; (b) concentration of Magainin I, 0.01 mg·mL^{-1}; (c) reaction temperature, 35 °C; (d) reaction incubation time, 35 min; (e) pH value, 6.0 and (f) concentration of NaOH, 4 M. Detailed steps and figures can be found in the Electronic Supporting Material.

Sensitivity of the assay

To assess the responses of the immunosensor based on the hand-held H_2 detector, various concentrations of *E. coli* O157:H7 were further tested. The H_2 concentration inside the confined space gradually increased due to dehydrogenation of AB, and H_2 was accurately measured by the hand-held detector. The H_2 concentration increased gradually with concentration of *E. coli* O157:H7, and there was a linear relationship in the range of 10 to 10^4 CFU·mL^{-1} between H_2 concentration and target *E. coli* O157:H7 concentration (Fig. 3). The LOD was 10 CFU·mL^{-1}. The linear regression equation was $Y = 0.4412X + 1.112$ ($R^2 = 0.989$) where Y is the relative aerogenesis intensity, X represents the common logarithm of the target bacteria concentration, and R^2 is the correlation coefficient. The comparative LOD between our established immunosensor and other reported *E. coli* O157:H7 methods is shown in Table 1. In contrast to other methods, the immunosensors achieved excellent performance for detecting *E. coli* O157:H7, with an LOD of 10 CFU·mL^{-1}. The lower LOD of the immunosensor may be attributed to the production of more H_2 by PtNPs catalyzing AB and high sensitivity of the hand-held H_2 detector. Compared with the traditional methods, such as fluorescence, electrochemistry and chemiluminescence, the approach did not rely on professional and expensive instruments.

Table 1 Comparison of different POC techniques for detection of *E. coli* O157:H7

| Analytical method	Linear range (CFU·mL^{-1})	Detection limit (CFU·mL^{-1})	Reference
Electrochemical biosensor	10^1–10^7	10^2	[25]
Electrochemical impedance spectroscopy	10^3–10^7	10^3	[26]
Con A-HRP-carbon nanotube-based ELISA	10^2–10^5	10^2	[27]
Electrochemical ELISAs	10^3–10^8	10^3	[28]
Quartz crystal microbalance	0–2.0×10^3	400	[29]
Chemiluminescence immunoassay	4.3×10^3–4.3×10^5	1.2×10^3	[30]
Our method	10–10^4	10	
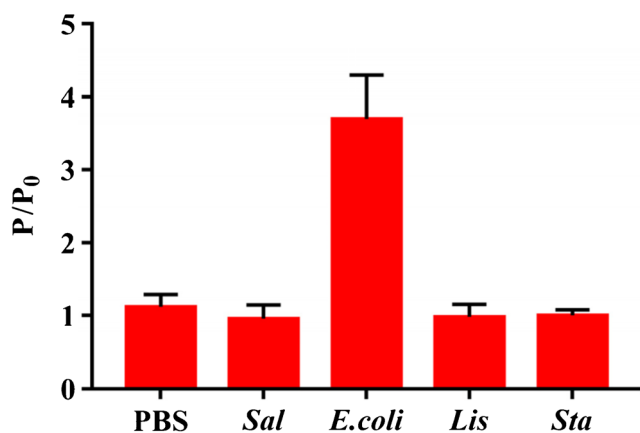
Fig. 4 Selectivity of the assay for detection of different targets

Specificity

To estimate the specificity of the method, the target *E. coli* O157:H7 and other nontarget pathogenic bacteria such as *Salmonella* sp., *S. aureus*, and *L. monocytogenes* were compared. The P/P_0 ratios of other bacteria were similar to those of the control group, while the P/P_0 ratio of *E. coli* O157:H7 was (Fig. 4). It is obvious from the results of these experiments that the method based on nanocomposites and hand-held H_2 detector was specific to *E. coli* O157:H7.

Reproducibility of this method

To verify applicability and selectivity capability of our method for detection of *E. coli* O157:H7, we tested the assay in spiked commercial milk samples. Milk samples were mixed with PBS containing different concentration of *E. coli* O157:H7. The recovery and relative standard deviation (RSD) of *E. coli* O157:H7 added to milk samples were in the range of 86–126% and 3–27%, respectively (Table S1).

Conclusions

We have demonstrated a specific, sensitive and immunosensor system approach based on H_2 generation in PPNs and a hand-held H_2 detector for portable detection of pathogenic bacteria. *E. coli* O157:H7 was detected quantitatively through a direct hand-held H_2 detector reading. These results show that the described assay is suitable for detection of a broad range of pathogens in real samples. The outstanding performance of the immunosensor approach was due to the high catalytic activity of PPNs in the dehydrogenation of AB, high H_2 capacity of AB, and high sensitivity of the hand-held H_2 detector. However, the practical application of this pathogenic bacteria detection strategy is still challenging, and strategies that are more efficient are required to simplify the experimental procedure. Nevertheless, the sensing platform POC detection of pathogenic bacteria based on the hand-held H_2 detector has potential for the future in terms of portable application in low-resource settings.

Compliance with ethical standards The authors declare no competing financial interest.

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