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Enhanced sensitivity of lateral-flow test strip immunoassays using colloidal palladium nanoparticles and horseradish peroxidase

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ABSTRACT

Although lateral-flow test strip (LFTS) immunoassays are rapid and require no specialized equipment, they are less sensitive than culture and polymerase chain reaction (PCR)-based methods for detecting bacteria. This study compared the sensitivity of LFTS assays based on colloidal palladium nanoparticles (PdNPs) and colloidal gold nanoparticles (AuNPs). PdNPs demonstrated a time and concentration dependent oxidation of the horseradish peroxidase (HRP) substrate 3,3′,5,5′-tetramethylbenzidine (TMB), whereas AuNPs did not. PdNPs labeled HRP-conjugated antibody lateral flow test strip (PdNPs-HRP LFTS) assays were tested with 3,3′-diaminobenzidine (DAB), a water insoluble substrate. The sensitivity of PdNPs-HRP LFTS assays in detecting *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Yersinia enterocolitica* was 5–10-fold higher than that of AuNPs-based LFTS (AuNPs LFTS) assays. PdNPs-HRP LFTS assays of reduced milk inoculated with *L. monocytogenes* were more than 10-fold more sensitive than conventional AuNPs LFTS assays. These results suggest that the PdNPs-HRP LFTS immunoassay is a promising tool for ensuring food safety.

1. Introduction

Food poisoning caused by bacteria remains a health problem. About 1500 outbreaks per year of *Listeria monocytogenes* occur in Europe (Barbau-Piednoir, Botteldoorn, Yde, Mahillon, & Roosens, 2013). In the United States, 390 outbreaks of *Escherichia coli* O157 were reported between 2003 and 2012 (Heiman, Mody, Johnson, Griffin, & Gould, 2015). Furthermore, outbreaks of yersiniosis caused by *Yersinia enterocolitica* have occurred in the United States, Japan and Norway after 2000 (Gupta, Gulati, Bhagat, Dhar, & Virdi, 2015).

Most of these outbreaks are caused by consumption of contaminated foods (Brasileiro et al., 2016; Jami, Ghanbari, Zunabovic, Domig, & Kneifel, 2014; Pennington, 2014; Rahman, Bonny, Stonsaovapak, & Ananchaipattana, 2011; Wang et al., 2015; Zunabovic, Domig, & Kneifel, 2011). To prevent the distribution of contaminated foods, manufacturers periodically assay for the presence of foodborne pathogens. Bacterial culture is the standard method, assaying for colony formation on selective media (Fukushima, Shimizu, & Inatsu, 2011; Välimaa, Tilsala-Timisjärvi, & Virtanen, 2015). Several days, however, are required to obtain results (Gupta et al., 2015). PCR-based methods, in which specific regions of genomic DNA are amplified and the generation of PCR products assessed by fluorescence (Abdollahzadeh, Ojagh, Hosseini, Irajian, & Ghaemi, 2016; Barbau-Piednoir et al., 2013; Garrido-Maestu, Chapela, Vieites, & Cabado, 2014; Välimaa et al., 2015) or gel electrophoresis (Tominaga, 2006, 2007), can be used to detect certain types of bacteria associated with food-poisoning. Although these methods are rapid, they require expertise and expensive equipment, making them unsuitable for on-site tests (Shan, Lai, Xiong, Wei, & Xu, 2015).

Lateral-flow test strip (LFTS) immunoassay is a rapid method suitable for on-site testing (Dzantiev, Byzova, Urusov, & Zherdev, 2014; Kim, Lim, & Mo, 2015). In this method, a suspension of food is applied to a sample pad containing antibodies (Abs) labeled with colloidal gold nanoparticles (AuNPs). Bacteria from the food suspension bind to the AuNP-labeled Abs to form a complex. Then, the complexes migrate into the test strip by capillary force and are captured on a specific strip by other Abs immobilized on the strip. The accumulation of AuNPs on the strip produces a visible red line. Although the test takes only about 15 min and requires no special equipment, its sensitivity is much lower than culture or PCR-based methods (Cho & Irudayaraj, 2013).

Here, an LFTS based on colloidal palladium nanoparticles (PdNPs) was developed. Pd catalyzes the oxidation of 3,3′,5,5′-

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tetramethylbenzidine (TMB) and 3,3′-diaminobenzidine (DAB); these substrates are also recognized by the enzyme horseradish peroxidase (HRP) in the presence of hydrogen peroxide (H_2O_2). Oxidation of DAB results in the formation of a dark brown precipitate. Additionally, aggregation of colloidal PdNPs results in the formation of a black precipitate in a manner similar to the red precipitate formed by colloidal AuNPs. The ability to form colored complexes by two different mechanisms should increase the sensitivity of antibody assays that utilize PdNPs as opposed to AuNPs. PdNPs labeled Abs and PdNPs and HRP double labeled Abs were compared to AuNPs labeled Abs using a LFTS platform.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The strains used in this study were *L. monocytogenes* ATCC 23073, *L. monocytogenes* ATCC 7645, *L. monocytogenes* ATCC 53979, *L. monocytogenes* ATCC 19112, *L. monocytogenes* ATCC 4428, *L. monocytogenes* ATCC 19114, *L. monocytogenes* ATCC 49592, *L. monocytogenes* ATCC 23074, *L. monocytogenes* ATCC 19115, *L. monocytogenes* ATCC 51781 and *L. monocytogenes* ATCC 9525. All L. *monocytogenes* strains were grown in brain heart infusion broth (Oxoid, Hampshire, United Kingdom) at 37 °C.

2.2. Materials

Heat-killed *E. coli* O157:H7, *L. monocytogenes* and *Y. enterocolitica* were purchased from Kirkegaard & Perry Laboratories (KPL), Inc., (Gaithersburg, MD, USA). Purified polyclonal antibodies (pAbs) to *E. coli* O157:H7, genus *Listeria* and *Yersinia* species were purchased from KPL. Nitrocellulose membrane HiFlow Plus HFB07504, cellulose fiber pads, and glass fiber pads were obtained from Millipore Corporation (Darmstadt, Germany). Solutions of AuNPs and PdNPs were purchased from Winered Chemical Corporation (Tokyo, Japan). These materials were described as being produced by reduction of amino acids or oligopeptides (Watabe, 2005). Peroxidase labeling kits − NH_2 were from Dojindo Molecular Technologies (Rockville, MD, USA). Metal enhanced DAB substrate kits were from Thermo Scientific (Waltham, MA, USA), and SureBlue™ TMB microwell peroxidase substrate was from KPL.

2.3. Development of LFTS

A nitrocellulose membrane was cut into strips, 5 mm in width and 25 mm in length. An absorption pad, measuring 10 mm in width and 20 mm in length, was placed downstream of the membrane. Capture pAbs (0.50 μg/test) were immobilized onto the membrane at a position 10 mm from the edge of the membrane at the side where the sample solution was dropped. Detection pAbs (0.25 μg/test) were labeled with AuNPs or PdNPs, mixed with 1.0×10^6 to 1.0×10^8 *L. monocytogenes* cells, 1.0×10^2 to 1.0×10^6 *E. coli* O157:H7 cells, or 5.0×10^5 to 5.0×10^7 *Y. enterocolitica* cells in 0.5 g skim milk l^{-1}, and applied to a conjugate pad measuring 5 mm in width and 10 mm in length. 60 μl of 0.5 g skim milk l^{-1} was used as the washing buffer. After 15 min, the strips were scanned and positive band intensity (signal values: a.u.) was measured. The bottom, plastic-coated side faced the light source of the scanner. Where indicated, 3,3′-diaminobenzidine (DAB) was applied to the membrane to generate colorimetric signals; otherwise, the signal was generated by precipitation of the colloidal Au or Pd. Each assay was performed in triplicate. A specific Ab capturing system designed to generate a control line to verify assay function and performance was not included during this early stage of development.

2.4. Conjugation of HRP to pAbs

HRP conjugation was performed using the Peroxidase Labeling Kit-NH2 (Dojindo Molecular Technologies, Inc.; Rockville, MD). Briefly, 100 μl of washing buffer containing 200 μg pAbs was added to a filtration tube. After mixing, the tube was centrifuged at 8000×*g* for 10 min. A 100 μl aliquot of washing buffer alone was added to the tube and was centrifuged at 8000×*g* for 10 min. Amine-reactive peroxidase dissolved in 10 μl of reaction buffer was then added to the filtration tube containing the pAbs and incubated for 2 h at 37 °C. Another 100 μl aliquot of washing buffer was then added to the filtration tube and was centrifuged at 8000×*g* for 10 min. Finally, 200 μl of storage buffer were added and the HRP-conjugated pAbs were recovered by pipetting.

2.5. 96-well plate assay

Aliquots of AuNPs (15 μl) or PdNPs (5, 10, and 15 μl) were suspended in 100 μl of SureBlue™ buffer containing TMB or 10 mM Tris/HCl (pH 9.0) buffer. The optical densities of the undiluted AuNPs solution and undiluted PdNPs solution were approximately 12. The mixtures were then added to each well of a 96-well plate, which was incubated at 37 °C with shaking at 200 rpm. Reactions were stopped by the addition of 100 μl 1 N HCl, and the absorbance (<lambda> = 450 nm) was measured using a microplate reader. The value obtained using Tris/HCl as a buffer was subtracted from that obtained using SureBlue™ as a buffer. Each assay was performed in triplicate.

2.6. Measurement of reflectance

A KPL product equivalent to 10^8 *L. monocytogenes* cells was mixed with detecting pAbs labeled with an equivalent amount of AuNPs- or PdNPs-labeled pAbs (AuNPs-pAbs or PdNPs-pAbs) and applied to commercially available test strips for *L. monocytogenes* (NH Immunochromato *Listeria*; NH Foods Ltd., Osaka, Japan). Fifteen minutes later, the substrate DAB was applied to the membrane, generating colorimetric signals. The reflectance of the membranes was then measured between 400 nm and 700 nm in increments of 1 nm using a UV-3600 spectrophotometer with a LISR-3100 integrating sphere attachment (SHIMADZU CORPORATION, Kyoto, Japan). A virgin test strip was used as a reference, and its measured value was set to 100%.

2.7. Food sample test

Reduced fat milk (low-fat milk) in liquid form was used as the sample matrix, essentially as described previously (Cho & Irudayaraj, 2013). Briefly, milk was inoculated at approximately 10^6 or 10^7 cfu/ml with *L. monocytogenes* (ATCC 53979), or used without inoculation (control). Colonies were grown on brain heart infusion agar and counted. The milk was mixed with detection Abs and then applied to LFTS, as described above. Each assay was performed in triplicate.

3. Results and discussion

3.1. Catalytic activity of PdNPs in solution

Incubation of PdNPs with SureBlue™ solution for 15–90 min resulted in the oxidation of TMB, yielding a blue color. The addition of HCl turned the solution yellow, and its absorbance was measured at 450 nm (Fig. 1). Absorbance was dependent on time and PdNPs concentration. In contrast, the addition of AuNPs did not oxidize TMB. SureBlue™ was designed to quantify the catalytic activity of