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Gold nanoparticle-based dynamic light scattering immunoassay for ultrasensitive detection of *Listeria monocytogenes* in lettuces

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ABSTRACT

Gold nanoparticle (GNP)-based dynamic light scattering (DLS) assay has been widely used for sensitive detection of small analytes based on analyte binding-induced GNP aggregation. However, the use of this new method to detect large biological objectives, such as pathogenic bacteria, has not been reported. This study is the first to describe a homogeneous GNP-based DLS immunoassay for ultrasensitive detection of *Listeria monocytogenes*. Compared with small analytes, *L. monocytogenes* has a larger surface and a higher number of antigen epitopes, which serve as carriers that bind to GNP probes to form "GNP-coated bacteria" complexes. To achieve better analytical performance, various parameters including GNP diameter and concentration, amount of labeled antibodies, and immunoreaction time were systematically investigated and optimized. Under the developed optimum conditions, limit of detection (LOD) for *L. monocytogenes* reached as low as 3.5×10^{1} CFU mL^{-1} in 0.01 M phosphate-buffered saline. Coupled with a large-volume immunomagnetic separation method, LOD for spiked lettuce samples reached 2.2×10^{1} CFU g^{-1}, which was one order of magnitude lower than the maximum limit imposed in Canada (100 CFU g^{-1}). The proposed method also exhibited excellent discrimination against 17 common pathogenic bacteria in lettuces. The developed GNP-based DLS immunoassay is highly promising as an approach for detecting large biological objectives.

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1. Introduction

Listeria monocytogenes is a Gram-positive, food-borne pathogen that can cause food poisoning even at a low infectious dose (~1000 cells) (Sharma and Mutharasan, 2013a, 2013b). Conventional culture-based method is usually regarded as the "gold standard" for detecting *L. monocytogenes*. However, the conventional method is time consuming (~7 d) and cannot provide on-site feedback (Lee et al., 2014). Various rapid detection methods, including polymerase chain reaction assay (Yang et al., 2007), enzyme-linked immunosorbent assay (Shim et al., 2008) and fluorescence biosensing (Wang et al., 2011) have been considered as potential alternative approaches to the conventional culture-based method for rapid screening of *L. monocytogenes*. However, limits of detection (LODs) of these approaches mostly range from 10^2 to 10^5 CFU mL^{-1} even when coupled with immunomagnetic separation (IMS). Thus, a rapid and sensitive method for detecting low levels of *L. monocytogenes* is needed.

Dynamic light scattering (DLS) is a powerful tool for determining the average hydrodynamic diameter (D_H) of particles ranging from 0.5 nm to 6 μm in a solution (Beqa et al., 2011; Dasary et al., 2010). Gold nanoparticles (GNPs) are routinely used as signal amplification probes for fabricating DLS-based biosensors (DLS-biosensors) because the light scattering ability of GNPs is higher than those of most biological samples (Jans and Huo, 2012). Recently, many DLS-biosensors have been investigated based on analyte binding-induced GNP aggregation assay or individual GNP size change upon analyte binding to sensitively detect small analytes, such as DNA, protein, and toxic metal ions (Jans and Huo, 2012). For example, Huo et al. described a GNP-based DLS-biosensor for the ultrasensitive detection of DNA with an LOD of 1 pM (Dai et al., 2008). Jans et al. reported a DLS-biosensor for the determination of human IgG using protein A-conjugated GNPs (Jans

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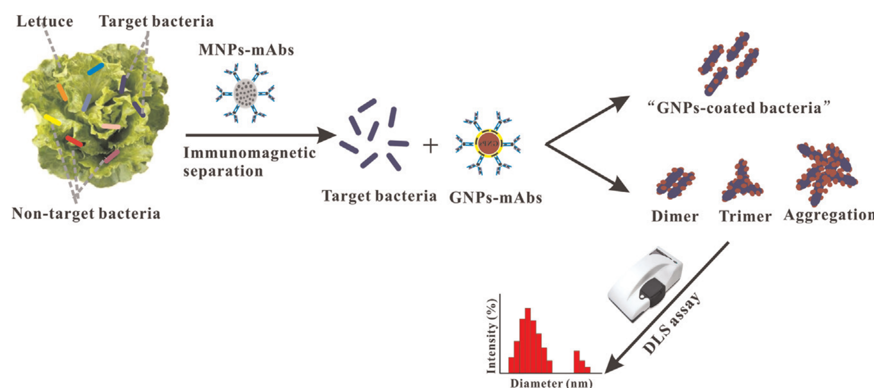

Scheme 1. Schematic representation of the proposed DLS assay format.

et al., 2009). Kalluri et al. used GNPs as probes in an ultrasensitive DLS assay for selective detection of arsenic in groundwater (Kalluri et al., 2009). However, a GNP-based DLS assay for rapid detection of large biological objectives, such as pathogenic bacteria, has not been reported as of this writing.

L. monocytogenes exhibit large isolate sizes (0.5 μm × 2.0 μm) and contain sufficient amount of antigen epitopes on their surfaces compared with small analytes. These properties make this species highly suitable for binding to GNP probes, thereby forming so-called "GNP-coated bacteria" complexes (Scheme 1). The Stokes–Einstein equation, i.e., $D_H = \zeta/3\pi \times \eta$ (where D_H is the hydrodynamic diameter, η is the viscosity of the solvent, and ζ is the friction constant), indicates that D_H significantly changes based on the size of the target analyte because ζ is proportional to the target radius. Scattering light intensity is also proportional to the 6th power of the target diameter, thereby sensitizing the DLS assay to the presence of large "GNP-coated bacteria" complexes at trace amounts. Thus, only a small amount of *L. monocytogenes* in the presence of GNP probe solution could induce a significant average D_H change. We hypothesize that GNP-based DLS assay would be a sensitive technique for quantitation of *L. monocytogenes* even at low concentrations.

This study is the first to report a GNP-based DLS immunoassay for rapid detection of pathogenic bacteria in lettuces. Various parameters including GNP diameter and concentration, amount of labeled antibodies, and immunoreaction time were systematically investigated and optimized. Combined with a large-volume IMS, the analytical performance of the proposed method was evaluated in terms of LOD, selectivity, and practicability using *L. monocytogenes*-spiked lettuce samples.

2. Materials and methods

2.1. Materials and instruments

Brain heart infusion (BHI) medium was purchased from Remel Microbiology Products (Lenexa, KS, USA), and PALCAM agar was obtained from Beijing Land Bridge Technology Co., Ltd. (Beijing, China). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC · HCl), N-Hydroxysulfosuccinimide sodium salt (NHSS), and bovine serum albumin (BSA) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Carboxyl-modified magnetic nanoparticles (MNPs; 180 nm) were purchased from Allrun Nano Science & Technology Co., Ltd. (Shanghai, China). Anti-*Listeria monocytogenes* monoclonal antibodies (mAbs, 5 mg mL^{-1}) were obtained from Jiangxi Zodolabs Biotech Corp. (Jiangxi, China). 0.01 M phosphate buffered saline (PBS) was prepared by adding 8 g of NaCl, 2.7 g of Na_2HPO_4, 0.2 g of KH_2PO_4, and 0.2 g of KCl into 1000 mL of Milli-Q water and was adjusted to pH 7.4 (unless otherwise specified) before use. All other reagents were analytical grade and purchased from Sinopharm Chemical Corp. (Shanghai, China)

A Malvern Zetasizer Nano ZS90 DLS system (Malvern Instruments Ltd., Worcestershire, UK) was used to conduct all DLS measurements. UV–vis absorption spectra of GNPs were obtained using an Amersham Pharmacia Ultrospec 4300 pro UV/visible spectrophotometer (England, UK). A high-resolution scanning electron microscope (SEM, Hitachi S-4800, Tokyo, Japan) and a high-resolution transmission electron microscope (TEM, JEOL JEM 2100, Tokyo, Japan) were used to determine the morphology of GNPs and the complex of anti-*Listeria monocytogenes* mAbs-coated GNPs and *L. monocytogenes*. Water purified by Elix-3 and Milli-QA (Molsheim, France) was used in all experiments.

2.2. Preparation of bacteria

L. monocytogenes (ATCC13932) cultures, which were stored at −70 °C as glycerol stock, were revived and maintained in BHI broth, and then grown overnight at 37 °C for activation. *L. monocytogenes* inoculum was prepared by sub-culturing an overnight culture plate into 5 mL of Luria–Bertani media containing 5 g L^{-1} yeast extract, 10 g L^{-1} tryptone, and 10 g L^{-1} NaCl, and then aerobically grown in an incubator shaker at 37 °C for 18 h with shaking at 180 rpm. Then, 0.1 mL of cultured samples was spread plated on the selective PALCAM plates incubated overnight at 37 °C for 24 h. Colony forming units (CFUs) on the agar plates were counted in duplicate as approximately 10^8 CFU mL^{-1}. Subsequently, *L. monocytogenes* cultures were harvested by centrifugation at 5000 rpm for 20 min at room temperature. Cells were washed thrice with PBS and then re-suspended in PBS to the required dilution. The *L. monocytogenes* suspension was 10-fold serially diluted with PBS to a final concentration from 10^0 to 10^8 CFU mL^{-1} before use. The obtained *L. monocytogenes* cells were killed by boiling in a 90 °C water bath for 15 min and stored at 4 °C for further use.

2.3. Preparation of GNPs

GNPs with small size were synthesized according to the citrate reduction method described by Li et al. (2013). Briefly, 100 mL of 0.01% gold chloride trihydrate solution in ultra-pure water was heated to boiling and 1.0 mL of 1% sodium citrate solution was added under constant stirring. When the color of the mixture changed to red purple, the solution was kept at boiling temperature for another 10 min. The obtained colloidal gold solution was stored at 4 °C for further use.

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