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Gold nanoparticle-based inductively coupled plasma mass spectrometry amplification and magnetic separation for the sensitive detection of a virus-specific RNA sequence

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ABSTRACT

We have developed a sensitive gold nanoparticle (AuNP)-based inductively coupled plasma mass spectrometry (ICP-MS) amplification and magnetic separation method for the detection of oligonucleotide sequences. The assay relies on (i) the sandwich-type binding of two designed probe sequences that specifically recognize the target oligonucleotide sequences, (ii) magnetic bead separation, and (iii) AuNP-based ICP-MS amplification detection. To enhance the analytical signal and minimize the background signal resulting from nonspecific binding, we performed a series of experiments to evaluate the effects of various parameters (the concentration of the capture probe; the time required for hybridization; the number of washings required to eliminate nonspecific binding) on the oligonucleotide detection. Under the optimized conditions, the detection limit was 80 zmol (corresponding to 1.6 fM of the target sequence in a sample volume of 50 μL). Moreover, it employs a shorter hybridization step and ICP-MS, this procedure is relatively simple and rapid (ca. 1.5 h). Based on the analytical results obtained using complementary and mismatched sequences, our method exhibits good performance in distinguishing complementary and random oligonucleotides. Compared with the "gold standard" methodology (plaque assay) for the quantification of dengue virus, our method has the capability to allow early detection of dengue virus in complicated and small-volume samples, with high specificity, good analytical sensitivity, and superior time-effectiveness.

1. Introduction

Disease-causing microorganisms (pathogens) can be classified as viruses, bacteria, fungi, or protozoan parasites. When the pathogen comes into intimate contact with living host tissue, a variety of diseases may arise through its adhering to and colonizing cell surfaces, invading tissues, and producing toxins and other harmful metabolic products; such pathogen-induced diseases may occur gradually while the pathogens proliferate to dangerous levels in the human body [1]. A positive correlation exists between the severity of pathogen-induced disease and the number of pathogens in the host [2]. To assess the disease status of patients infected by pathogens, the detection of the number of pathogens, characterized by their unique nucleic acid sequences, has become a popular molecular method for medical diagnosis. Several techniques are available for the detection of oligonucleotide sequences. In many of them, the achievable sensitivity of the method depends crucially on the specific activity of the label moiety linked to the oligonucleotide probe. Many types of label moieties, including radioactive nuclides [3], fluorescent [4,5] and chemiluminescent compounds [6,7], have been used to facilitate sensitive oligonucleotide detection. Moreover, several promising label-free detection methods have also been developed to solve the problems associated with label-based detection methods [8]. According to the literatures, the progress in the label-free detection methods has been made in surface plasmon resonance- and bioluminescence-based optical detection [9,10], nanowire-based electrical [11] and electrochemical measurements [12].

Recently, metal and semiconductor nanoparticles (NPs) have emerged as promising tags for the detection of oligonucleotide hybridization because of their unique optical and electrical properties, including large extinction and scattering coefficients, catalytic activity, surface electronic activity, and efficient Brownian motion in solution [13,14]. Modification of oligonucleotides with AuNPs for the detection of DNA fragments was first reported by Mirkin and co-workers over 10 years ago [15]. This colorimetric detection

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technique is relatively simple and rapid and requires no expensive instrumentation. Moreover, it exhibits a peculiar characteristic of sharp melting transitions that can be applied to differentiate full complementarity from single-nucleotide polymorphism [16]. Nevertheless, even when larger NPs (50–100 nm), which have greater molar extinction coefficients, are employed, the achievable sensitivities of this nanoparticle–oligonucleotide detection scheme are poorer than those of conventional fluorophore-based assays (typically in the picomolar range; the best reported detection limit is ca. 600 fM) [17]. To improve the analytical sensitivity, various techniques – including fluorescence [18], resonant light-scattering [19,20], scanometric [21], and Raman [22] spectroscopies – have been employed to amplify the analytical signal. Moreover, the detection limit can be lowered to the attomolar level when using silver stain combined with bio-barcode amplification techniques [23]. Notably, however, as described by Dai et al. [24], all of these amplification methods involve complicated multiple-step procedures that not only are time-consuming but also often cause problems in reproducibility.

In addition to the use of fluorescent tagging and silver stain techniques, a variety of NPs have been widely employed as labels for the amplified detection of oligonucleotides to improve the analytical sensitivity and the simplicity of the method. Electrochemical [25] and chemiluminescent [26] detection techniques are all sensitive, simple, inexpensive, and rapid procedures for the detection of oligonucleotide hybridization from the signals provided by metal NP labels.

In a manner similar to the fluorescence-labeling method, the potential of using element tags coupled with ICP-MS for the immunoassay has been identified [27]. As stated by Tanner et al. [28], for affinity-based assays, ICP-MS can also provide advantages over other techniques in terms of high sensitivity, large dynamic range, high precision, high throughput, lower matrix effects, lower background from plastic containers and plates, larger multiplexing potential, and better spectral resolution sensitivity. By way of determining labeled AuNPs, Zhang et al. [29], Baranov et al. [30], and Quinn et al. [31] have all developed immunological methods based on the coupling of sandwich-type immunoreactions with ICP-MS. Merkoçi et al. [32] also used ICP-MS in the immunoassay of peptide-conjugated DNA; this approach required a suitable peptide sequence covalently linked with an oligonucleotide and the use of antibodies. For all of the methods described above, either a nitrocellulose membrane or a 96-well microtiter plate was used as the flat substrate to capture the target. Consequently, in view of the limited specific areas provided by flat substrates, these approaches may provide poor analytical sensitivity because of the slower target-binding kinetics and the formation of lower amounts of sandwich structures on such substrates [17,33].

To improve the binding kinetics between the capture and target oligonucleotide sequences and thereby enhance the analytical sensitivity, Wu et al. [34] used magnetic microparticles (MMPs), instead of a flat substrate, to push the equilibrium between the oligonucleotide sequences toward their hybridization by increasing the concentration of MMP probes; this approach cannot be done in heterogeneous assays. Moreover, Wu et al. also attempted to use larger silica nanoparticles (SiNPs, 400 nm) as tags to improve the signal intensity through more-intense amplification of the signal. Nevertheless, the lower collision probability of the SiNPs, the lower hybridization probability, and the inferior sensitivity of inductively coupled plasma optical emission spectrometry (ICP-OES) resulted in the lowest concentrations of DNA detected when using AuNPs and SiNPs being only 350 and 3.5 pM, respectively. To improve the analytical sensitivity and selectivity, recently, Le et al. [35] employed similar procedure and ICP-MS to analyze thrombin using aptamer modified MMP and AuNP as scaffold and label. According to Le [35], the detection limit can be achieved as low as 0.5 fmol, corresponding to 10 pM thrombin in 50 μL.

According to Scheffer et al., [36,37] in light of the analytical benefits of ICPs (high sensitivity and precision; quantitative and qualitative information about the elemental content of a sample), it is likely that more bionanotechnological applications will be developed based on ICPs. Nevertheless, the detection limits obtained using ICP-OES – and ICP-MS – based oligonucleotide detection methods are all in the picomolar range [32,34]. Currently, RT-PCR (reverse transcription-polymerase chain reaction) is the most widely used and sensitive method for the detection of RNA sequence levels. However, for conventional RT-PCR technique, it is indispensable to extract the sequence from the sample matrix (usually requires 2 h) in advance. Otherwise, the analytical results may be severely interfered with the matrix (cell fragment or protein) during the amplification and detection steps. Therefore, to develop a sensitive, simple and time-saving alternative to typical RT-PCR detection schemes for the quantitative determination of target oligonucleotides is the main aim of this work. To improve the simplicity and analytical sensitivity of ICP-MS – based oligonucleotide detection methods, in this study we developed a method employing (i) MMP probes to homogeneously separate the target oligonucleotide sequence from the sample matrix and (ii) the amplification effect of measuring labeled AuNPs, which release a large numbers of Au ions during ICP-MS measurement, for the detection of the target oligonucleotide.

2. Experimental

2.1. Instrumentation

The determination of Au was performed using a quadrupole ICP-MS (Agilent 7500a, Agilent Technologies). The instrumental operation conditions selected for the Au determination are listed in Table S1. Absorbance measurements of oligonucleotides and AuNPs were collected using a Helios Delta UV–vis spectrophotometer (Thermo Fisher Scientific). Transmission electron microscopy (TEM) images were recorded using a JEOL 2011 electron microscope operated at an acceleration voltage of 200 keV.

2.2. Chemicals and materials

AuNPs, solvents and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). NAP-5 columns were obtained from GE Healthcare (Piscataway, NJ, USA). Ultrapure nitric acid and ultrapure hydrochloric acid (J. T. Baker, Phillipsburg, NJ, USA) were used to prepare the aqua regia solution for dissolving the AuNPs. Succinimidyl-4-(*p*-maleimidophenyl)butyrate (SMPB) and sulfosuccinimidyl acetate (Sulfo-NHS-acetate) were obtained from Pierce Biotechnology (Rockford, IL, USA). The solution of amino-functionalized MMPs (Dynabeads® M-270 Amine) and the magnetic particle concentrator (Dynal MPC™-S) were obtained from Invitrogen (Carlsbad, CA, USA). Oligonucleotide probes were designed using the program Primer3 [38] and purchased from Integrated DNA Technology. (Coralville, IA, USA). For the detection of DENV2 (GenBank EF540856), a 113-base-long viral RNA fragment (5′-*GCA CUG UCA CGA UGG* AGU GCU CUC CGA GAA CGG GCC UCG ACU UCA AUG AGA UGG UGU UGC UGC AAA UGG AAA AUA AAG CUU GGC UGG UGC ACA GGC AA*U GGU UCC UAG ACC UG*-3′) at the domain of the E gene was selected as the target (Fig. 1). The specific capture probe (5′-HS-$(CH_2)_6$-$(A)_{12}$-CAG GTC TAG GAA CCA-3′) and report probe (5′-CCA TCG TGA CAG TGC-$(A)_{12}$-$(CH_2)_3$-SH-3′) were designed to be complementary to the two ends of the target (italic regions). For optimization experiments, a 30-mer mock oligonucleotide target (5′-GCA CTG TCA

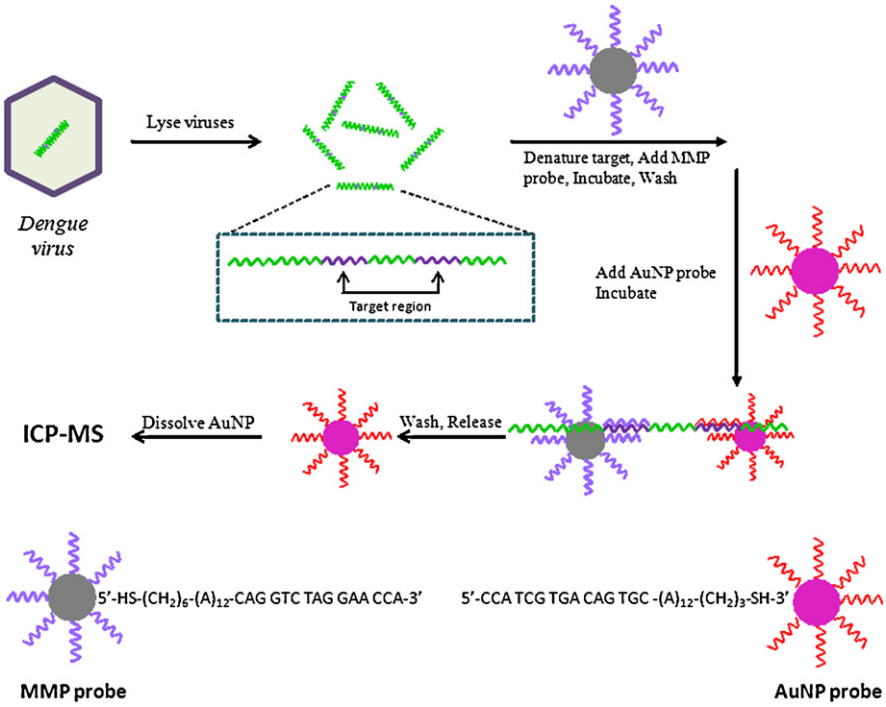

Fig. 1. AuNP-based "sandwich-type" oligonucleotide detection by ICP-MS. The viral RNA sequences were obtained by directly lysing the viruses. After denaturing the RNA sequences, the MMP probes were added to capture and isolate the target oligonucleotide from sample matrix. The signal amplifiers, "AuNP probes," were then added to form a sandwich structure with the MMP–target oligonucleotide complexes. After removing the unbound AuNP probes, the AuNP probes on the MMP surface were thermally released. Finally, the AuNP probes were dissolved in 2% aqua regia and measured using ICP-MS.

CGA TGG TGG TTC CTA GAC CTG-3′) that was fully complementary to the two probes was designed according to the italic domains to mimic the oligonucleotide fragment [39]. In addition, the negative control sequence was a random sequence (5′-AGC ATG GTC GAT AGG AAA CGA CTC TAG CGC-3′) that was noncomplementary to either the capture or report probes.

2.3. Preparation of AuNP probes

AuNP probes were prepared according to literature procedures [40]. Dithiothreitol (DTT) solution (0.1 M) was prepared in cleavage buffer (0.17 M phosphate buffer, pH 8.0); the disulfide bond of the oligonucleotide was cleaved with DTT solution (100 μL) for 3 h. To purify the thiolated oligonucleotide, a NAP-5 column (GE Healthcare, Piscataway, NJ, USA) was used to separate the thiolated oligonucleotide from the excess DTT and the salt matrix according to the protocol supplied by the manufacturer. The freshly reduced report probe (2 nmol) was added to the AuNPs (20 nm, 1 nM, 1 mL) and shaken gently overnight. The system was buffered to a phosphate concentration of 10 mM (pH 7.0), including 0.01% SDS. Over the course of 2 days, the solution was brought to 0.3 M NaCl in a stepwise manner through the addition of 2 M NaCl. Prior to use, the NPs were spun (8000 rpm) and rinsed four times with assay buffer (10 mM sodium phosphate, 0.15 M NaCl, 0.01% SDS, pH 7.4) to remove unbound oligonucleotide.

2.4. Preparation of MMP probes

The amino-functionalized MMP probes were modified with the capture probe according to procedures developed by the Mirkin group [41]. MMPs (1 mL, 30 mg mL^{-1}) were dispersed in a solution of SMPB (50 mg) in anhydrous DMSO (15 mL) and rotated for 4.5 h. The beads were then washed with anhydrous DMSO and coupling buffer (0.2 M NaCl, 0.1 M sodium phosphate, pH 7.0). After reaction with the capture probe (300 μL, 10 μM) overnight, the beads were passivated with sulfo-NHS-acetate (100 mg) in passivation buffer (35 mL, 0.15 M NaCl, 0.15 M sodium phosphate, pH 8.0) for 1 h. After washing with the passivation buffer and assay buffer, the MMPs were finally re-dispersed in assay buffer (3 mL, to give a final concentration of 10 mg mL^{-1}) and stored at 4 °C. The coupling efficiency was calculated by measuring the change in the oligonucleotide concentration before and after the reaction. There were approximately 4.35×10^5 capture probes per MMP.

2.5. Detection procedure

This assay was conducted using a typical sandwich assay format (Fig. 1). In a typical experiment, the MMP probes (20 μL, 40 mg mL^{-1}) were placed in a 1.5-mL microcentrifuge tube; after removing its supernatant, the mock target oligonucleotide (50 μL) was heated (90 °C) for 10 min and then mixed with the MMP probes. Serial dilutions of the mock target were detected in parallel during a typical experiment. The hybridization time between the target and the MMP probes was set for 1 h at 37 °C under gentle rotation. After washing twice with assay buffer (1 mL), the MMP–target complex was resuspended in AuNP probes (0.2 nM, 50 μL) and left to hybridize with them for 30 min at 37 °C. The resulting mixture was then washed with assay buffer (9 mL × 1 mL). Following the final washing, the mixture was moved to a new tube (to prevent a background signal resulting from nonspecific binding of the oligonucleotide-conjugated AuNPs to the interior of the used tube) and resuspended in deionized water (100 μL) and left to incubate for 10 min at 60 °C. Finally, the solution containing the released AuNP probes was drawn out and mixed with aqua regia [2% (v/v), 100 μL]. The dissolved Au ions were then introduced into the ICP-MS for detection at m/z 197. During analysis of the cell culture supernatants, the viral RNA sequences were obtained by directly adding lysis buffer [0.1% n-octyl-β-D-glucoside (final concentration), MP Biomedicals, Irvine, CA, USA] to lyse the viruses present in the medium, performing a 5-fold dilution with assay buffer,

and subsequent assaying using the detection procedure described above.

2.6. DENV preparation

A local isolated dengue virus serotype 2 (DENV2, PL046) was supplied by Professor Yun-Liang Yang (National Chiao Tung University, Hsinchu, Taiwan). The DENV was transfected and amplified in mosquito cells, *Aedes albopictus* C6/36 (ATCC number CCL 126^{TM}), which had been obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). C6/36 cells were grown in minimum essential medium (MEM, Gibco, Grand Island, NY, USA) and supplemented with 0.22% sodium bicarbonate (Sigma), 1% penicillin/streptomycin (PS, Gibco, Grand Island, NY, USA), and 10% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) at 28 °C in a humidified incubator under an atmosphere of 5% CO2. The viruses were harvested from the supernatants and stored at −80 °C prior to use. Each virus titer was determined using plaque assays.

2.7. Plaque assay of DENV

The plaque assay of DENV was performed using BHK-21 cells (ATCC number CCL-10). BHK-21 cells (10^5) suspended in MEM, supplemented with 0.22% of sodium bicarbonate, 1% PS, and 5% heat-inactivated FBS, were seeded into each well of a 24-well culture plate and incubated at 37 °C in a humidified incubator under an atmosphere of 5% CO2. Virus serial tenfold dilutions (10^3–10^8, 100 μL) were added to each well when the cells were cultured overnight. After an incubation period of 1 h, 1 mL of the MEM (containing 1% carboxylmethyl cellulose, 1% PS, and 5% FBS) was added and the plates then incubated for 5–7 days at 37 °C. The plaque-forming wells were fixed with 3.7% formaldehyde for 30 min, followed by staining with 0.5% crystal violet in 3.7% formaldehyde. The results were determined by counting plaques in three replicate wells.

3. Results and discussion

3.1. Characterization of MMP/Target/AuNP complexation

To demonstrate the feasibility of this nanoprobe-based "sandwich-type" oligonucleotide detection strategy, we used TEM to image the sandwich hybridization structure consisting of the target oligonucleotide hybridized to the MMP and AuNP probes. Fig. S1 presents a high-resolution TEM image of the sandwich structure; it clearly indicates that the AuNP probes were connected by the "double helix bridge" to an MMP surface to form a sandwich-like structure.

3.2. Optimization of sandwich hybridization assay

3.2.1. Concentration of MMP probes

To simplify the analytical procedure and improve the analytical sensitivity, ICP-MS was used for the final detection in this study. To avoid the interference effects resulted from the complicated sample solution containing 10% (w/v) protein and 0.15 M NaCl, we employed "sandwich-type" strategy to form MMP–target–AuNP complexes and thus we could easily use a magnet to isolate the complexes from the sample solution prior to AuNP probe releasing and ICP-MS detection. To maximize the analytical sensitivity, we investigated the concentration of MMP probes required for the formation of the MMP–target oligonucleotide complexes.

In this study, the target oligonucleotide and AuNP probe concentrations were 1 pM and 0.2 nM, respectively, and the hybridization time was set at 2 h; the negative control was performed using the same solution, which contained 60 mg mL^{-1} of the MMP probes, but no target oligonucleotide. Fig. S2 indicates that when the concentration of the MMP probes increased from an initial value of 10 mg mL^{-1}, the capture efficiency of the target oligonucleotide increased accordingly, leveling off when the concentration of the MMP probes exceeded 40 mg mL^{-1}. Because similar capture efficiencies could be achieved when 40 mg mL^{-1} and 60 mg mL^{-1} of MMP probes were used, to attain better capture efficiency of the target oligonucleotide and lower operation cost, we selected an MMP probe concentration of 40 mg mL^{-1} for our subsequent experiments.

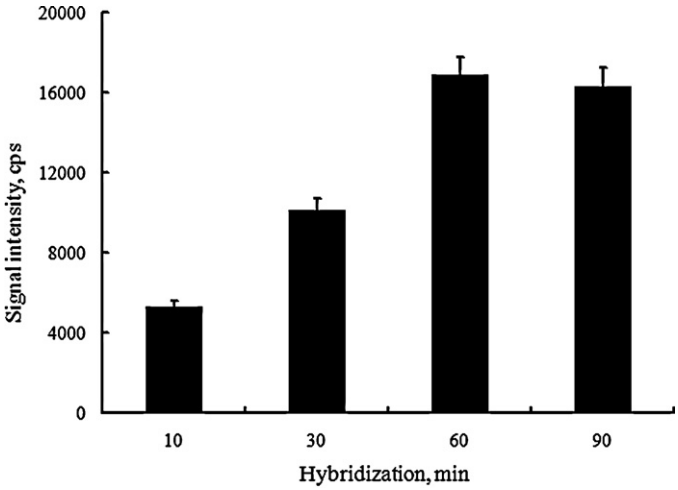

Fig. 2. Variation of the analytical signal with respect to the length of the hybridization time between the MMP probe and the mock oligonucleotide ($n = 3$). Concentrations of the MMP probes, target oligonucleotide, and AuNP probes: 40 mg mL^{-1}, 1 pM, and 0.2 nM, respectively.

3.2.2. Hybridization time

The time required for adequate probe–target hybridization depends upon the type of probe and the sensitivity required. To minimize the working time and maximize the analytical performance, we investigated the time required for the two hybridization reactions; i.e. the formation of the MMP–target oligonucleotide and AuNP–target oligonucleotide complexes.

To determine the optimal hybridization time for the MMP–target oligonucleotide reaction, a mixture containing 1 pM of the target oligonucleotide and 40 mg mL^{-1} of the MMP probes was left to react for periods of time ranging from 10 to 60 min prior to its mixing with the AuNP probes (0.2 nM). After 2 h of mixing the MMP–target oligonucleotide complex with the AuNP probes, we examined the variation in the analytical signal as a function of time for the hybridization of the target oligonucleotide to the MMP probes. Fig. 2 reveals that the signal intensity increased upon increasing the hybridization time, but it leveled off when the hybridization time was greater than 60 min. Thus, we selected a hybridization time of 60 min to ensure complete hybridization and thereby achieve maximum sensitivity in our subsequent experiments.

To ensure complete hybridization of the MMP–target oligonucleotide complex with the AuNP probes, we mixed the target oligonucleotide (1 pM) and the MMP probes (40 mg mL^{-1}) together for 60 min (to allow the complete formation of the MMP–target oligonucleotide complex) prior to their mixing with the AuNP probes (0.2 nM). To determine the optimal hybridization time for the MMP–target oligonucleotide complex with the AuNP probes, we examined the variation in the analytical signal as a function of time for the hybridization of the AuNP probes with the MMP–target oligonucleotide complex. Fig. 3 reveals that 30 min was sufficient to

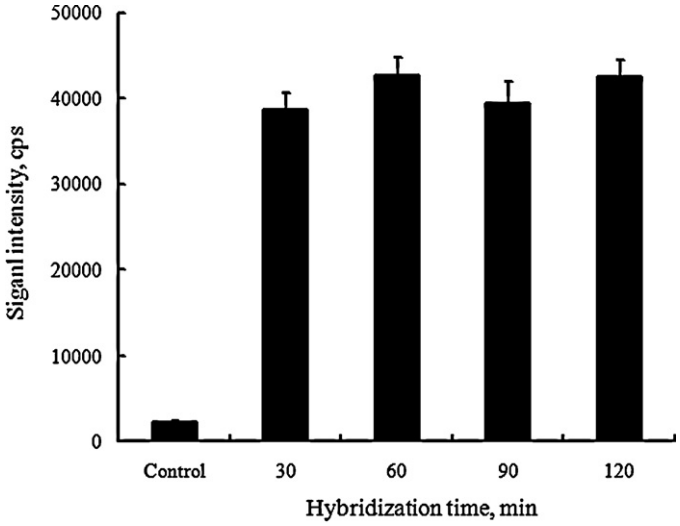

Fig. 3. Variation of the analytical signal with respect to the length of the hybridization time between the MMP–target oligonucleotide complex and the AuNP probes ($n = 3$). Concentration of MMP and AuNP probes: 40 mg mL^{-1} and 0.2 nM, respectively. Apart from the control sample, 1 pM of the target oligonucleotide was added in each sample.

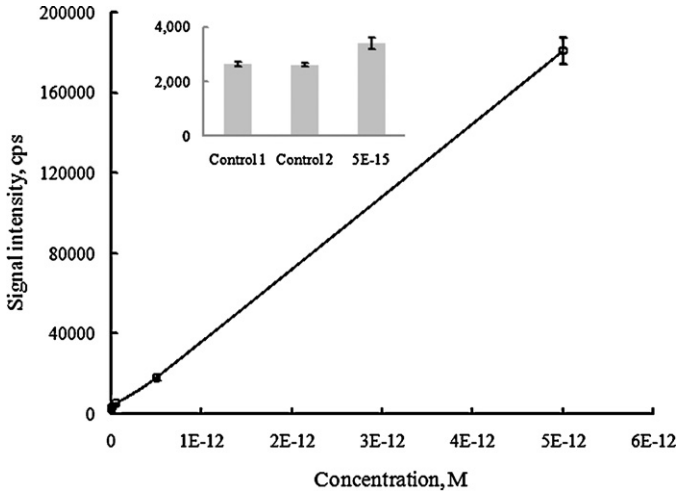

Fig. 4. Signal intensity plotted as a function of the concentration of the target sequence ($n = 3$). The samples for Control 1 and Control 2 were prepared with and without the addition of the random sequence, respectively. Concentrations of MMP and AuNP probes: 40 mg mL^{-1} and 0.2 nM, respectively. ($R^2 = 0.999$).

ensure complete hybridization of the MMP–target oligonucleotide complex with the AuNP probes. Although a shorter hybridization time was possible for the hybridization reaction, to ensure complete hybridization for samples containing lower concentrations of the target oligonucleotide (<1 pM), we selected a hybridization time of 30 min for the hybridization of the MMP–target oligonucleotide complex with the AuNP probes in our subsequent experiments.

3.2.3. *The effect of the number of washing*

In sandwich hybridization experiments, nonspecific binding of AuNP probes can lead to decreased detection sensitivity, thereby resulting in low signal-to-background (S/B) ratios and aberrant final results. According to Selvaraju et al. [42], the absence of washing leads to a very high background signal, arising from high nonspecific binding. Fig. S3 displays the variation in the signal intensities obtained with and without target oligonucleotide at different number of washings. We observe that the background signals (the inset) obtained without target oligonucleotide decreased with increasing number of washings. Because there was only about 2.77% of the signal obtained with target oligonucleotide might lose after each washing step, we used assay buffer to wash the MMP–target oligonucleotide and AuNP–target oligonucleotide complexes for 9 times to minimize the background signal and maximize the analytical signal in our subsequent experiments.

To clarify the causation of background signal, we added different amount of MMP probe (2.5–60 mg mL^{-1}) to a negative control solution, which contained 0.2 nM of AuNP probe, but no target oligonucleotide. In view of similar background signals (~1700 cps) were obtained at different amount of MMP probes, we conclude that the background signal should come from the AuNP nonspecific binding to the interior of tube instead of the surface of MMP. To reduce the background signal, we transferred the sandwich structures to a new tube after the final washing.

3.3. *Analytical performance*

Under the optimized conditions, the detection limit of the target oligonucleotide, measured at m/z 197 using a typical calibration curve approach, was 1.6 fM with a linear range from 5 fM to 5 pM (Fig. 4). The detection limit was reached based on three times the standard deviation of the background noise ($n = 7$), and the relative standard deviation (RSD) of variant target concentrations were in the range of 2.2–7.2%. To evaluate the detectability of this method, we assayed a positive control sample (containing 5 fM of complementary sequence) and two negative control samples (containing 1 nM of random (control 1) and no oligonucleotide (control 2) sequences, respectively). The inset to Fig. 4 reveals that only the complementary sequence provided an obvious signal compared with random sequences under the optimized conditions. In addition, a comparison of the signal intensities of the two negative control samples revealed that background signals resulting from the occurrence of nonspecific binding, due to the presence of non-complementary sequences, was negligible. Moreover, to evaluate the matrix effects, 1 pM target oligonucleotide were spiked to an assay buffer containing 2% BSA (w/v) and a 5-fold diluted MEM (containing 10% FBS), respectively, and analyzed with our developed method. Based on the experimental results, the spike recoveries of the target oligonucleotide were 98.4% and 97.8%, respectively. In other words, the developed method should be useful for determining analyte oligonucleotides not only in pure assay buffer but also complex culture medium.

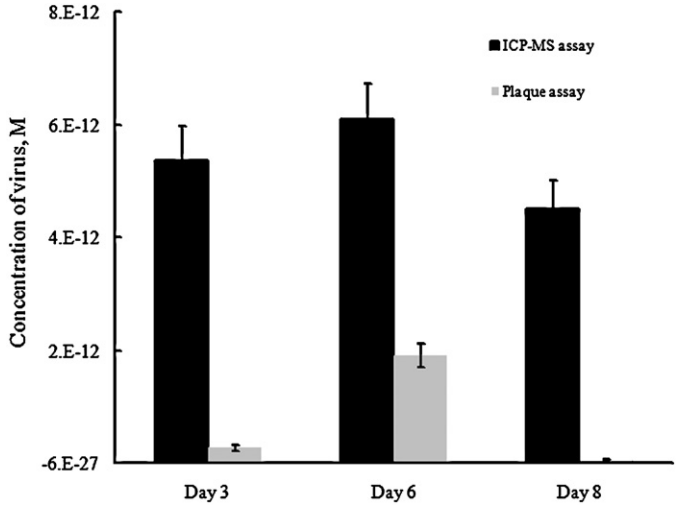

Fig. 5. Correlation between the concentrations of nucleic acid measured using the ICP-MS – based sandwich assay and the plaque numbers of DENV.

Table 1
Comparison of analytical characteristics between this and previous related studies.

Functional nanomaterial	Detection method[b]	Detection limit	Length of hybridization time	Reference
Au NPs (non-cross-linked; 15 nm)	Colorimetric	500 nM	<3 min	44
Au NPs (cross-linked; 13 nm)	Colorimetric	2–10 nM	Several tens of minutes	16
Ag NPs (cross-linked; 30 nm)	Colorimetric	40 pM	Several tens of minutes	43
Au and Si NPs (13 nm; 400 nm)	ICP-AES	350 pM (AuNP) 3.5 pM (SiNP)	ca. 5.5 h	34
Au NPs (12 nm)	SPR	10 pM	2 h	45
Au NPs (31 nm)	Fluorescence	7 pM	2 h	18
Au NPs (50 and 100 nm)	Light Scattering	1 pM	2 h	19
Au NPs (13 nm)	SERS	ca. 20 fM	3.5 h	22
Ag NPs (21 nm)	CL	5 fM	4 h	26
Si NPs (55 nm; HFNP)	Fluorescence	0.8 fM	–	48
Au NPs with Ag amplification (20 nm)	Electrochemical	32 pM	20 min	47
Au NPs with Ag amplification (13 nm)	Conductivity	500 fM	6 h	46
Au NPs with Ag amplification (13 nm)	Scanometric	50 fM	8 h	21
Au NPs with Ag amplification (30 nm; BCA)	Scanometric	500 zM	ca. 4 h	23
Au NPs (20 nm)[a]	ICP-MS	1.6 fM	1.5 h	–

[a] This method.
[b] SPR, surface plasmon resonance; SERS, surface-enhanced Raman scattering; CL, chemiluminescence; BCA, bio-barcode amplification; HFNP, highly fluorescent bioconjugated NPs.

3.4. Comparison of different methods

Table 1 compares the characteristics and performance of our developed method with those of other reported metal NP – based oligonucleotide detection methods. The detection limit of our developed system is lower than those obtained using colorimetric [16,43,44], fluorescence [18], light scattering [19], ICP-OES [34], surface plasmon resonance (SPR) [45], electric [46], and electrochemical [47] detection methods, and is comparable to those obtained using scanomeric [21], surface-enhanced Raman spectroscopy (SERS) [22], and chemiluminescence [26] methods. Notably, however, the detection limits of the methods described by Mirkin et al. (500 zM) [23] and Zhao et al. (0.8 fM) [48], employing bio-barcode amplification (BCA) and highly fluorescence bioconjugated NPs (HFNP) techniques, respectively, remain the lowest yet reported.

We suspect that our developed signal amplification method should be applicable to the determination of analyte oligonucleotides at levels lower than the femtomolar regime if we were to use larger AuNP labels as signal reporters. In addition to the excellent sensitivity achieved from the combination of AuNP labels and ICP-MS, our procedure is simple and time-saving (ca. 1.5 h), relative to typical sandwich-type detection schemes, because of the shorter hybridization time and the use of ICP-MS.

3.5. Application for DENV detection

According to Acosta et al. [49], DENV is the most significant mosquito-borne human pathogen; it is responsible for over 50 million infections of variable severity each year. To evaluate the applicability of this ICP-MS – based "sandwich" detection method, we attempted to correlate the number of a DENV2-specific sequence determined using our developed method with the results of a traditional plaque assay that is considered to be the "gold standard" methodology for the quantification of dengue virus [50]. According to Bae [51] and Baert [52], the virus genome numbers determined by real-time polymerase chain reaction (PCR) are, in general, significantly higher than the number of infectious particles determined by the plaque assay; the ratios of virus genomes to infectious particles were reported to be in the approximate range from 10^1 to 10^3. These findings arise because the plaque assay can determine only the number of infectious particles present in the media, whereas quantitative real-time PCR cannot distinguish between infectious and noninfectious viral genomes. Similarly, the titer of DENV2 measured by our developed method was about 10-fold higher than that determined by the plaque assay. Moreover, Fig. 5 reveals a satisfactory correlation ($R^2 = 0.81$) between the analytical results achieved using the two methods. Accordingly, we suspect that our proposed method is applicable to the early diagnosis of DENV2 and the viremia titers of infected patients [53] with high specificity, good analytical sensitivity, and superior time-effectiveness.

4. Conclusions

There is currently a need for time-effective and simple diagnostic methods – combining sensitivity, specificity, and accurate quantization capability – for the detection of pathogens via their unique nucleic acid sequences. Because our developed method relies on oligonucleotide-modified AuNPs for signal amplification and MMPs for ready separation, the use of AuNPs as labels in ICP-MS – based "sandwich" detection systems can allow the direct detection of low-femtomolar levels of oligonucleotides without the need for additional amplification processes. In addition to its high sensitivity, our developed method is a simple and time-saving alternative to typical sandwich-type detection schemes for the quantitative determination of target oligonucleotides, because it employs a shorter hybridization step and ICP-MS were used in the typical sandwich-type detection schemes. Compared with the "gold standard" methodology for the quantification of dengue virus, our method appears to be an accurate and rapid approach toward the early diagnosis of dengue virus infection. In other words, this highly sensitive and time-effective method might allow rapid clinical and epidemiological assessments, potentially translating into improved patient care and more timely community intervention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.02.005.

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