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Metal-doped inorganic nanoparticles for multiplex detection of biomarkers by a sandwich-type ICP-MS immunoassay

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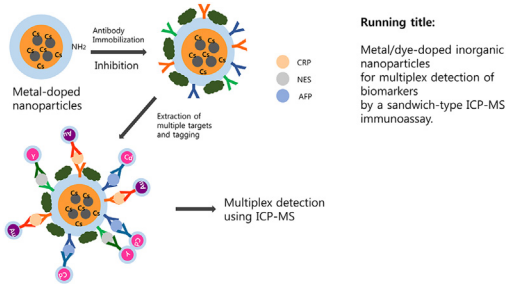
HIGHLIGHTS

- Metal-doped inorganic nanoparticles were synthesized for the multiplex detection of biomarkers.
- Cs-doped multicore magnetic nanoparticles (MMNPs) were synthesized for magnetic extraction.
- Three different metal/dye-doped silica nanoparticles (SNPs) were synthesized as probes for multiplex detection.
- Excellent LOD and recoveries were obtained for human serum samples with no sample treatment.
- The proposed method provided short analysis time and convenience for clinical application.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal-doped inorganic nanoparticles were synthesized for the multiplex detection of biomarkers by a sandwich-type inductively coupled plasma mass spectrometry (ICP-MS) immunoassay. The synthesized Cs-doped multicore magnetic nanoparticles (MMNPs) were used not only for magnetic extraction of targets but also for ratiometric measurement in ICP-MS. In addition, three different metal/dye-doped silica nanoparticles (SNPs) were synthesized as probes for multiplex detection: Y/RhBITC (rhodamine B isothiocyanate)-doped SNPs for CRP (cardiovascular disease), Cd/RhBITC-doped SNPs for AFP (tumor), and Au/5(6)-XRITC (X-rhodamine-5-(and-6)-isothiocyanate)-doped SNPs for NSE (heart disease). For quantification, the doped metals of SNPs were measured by ICP-MS and then the signal ratio to Cs of MMNPs was plotted with respect to the concentration of targets by a ratiometry. Limits of detection (LOD) of 0.35 ng/mL to 77 ng mL^{-1} and recoveries of 83%–125% were obtained for serum samples spiked with the biomarkers. Since no sample treatment was necessary prior to the extraction, the proposed method provided short analysis time and convenience for the multiplex determination of biomarkers, which will be valuable for clinical application.

1. Introduction

Some biomarkers are closely related with certain targeted diseases especially in tumor diagnosis and their elevated levels were

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associated with disease progression [1–5]. Since certain biomarkers are often related to several diseases, multiplex detection could enhance diagnosis accuracy, which inevitably requires labeling techniques using immunoreaction. Several detection methods have been reported [6–8], including electrochemical detection [9], chemiluminescence [10], and inductively coupled plasma mass spectrometry (ICP-MS) with element tagging [11,12]. Recently, inorganic nanoparticles such as quantum dots [13–15], gold nanoparticles [16,17], and silver nanoparticles [18] have received attention because they amplify the signal as a probe and improve the sensitivity and accuracy in ICP-MS measurement. However, because of a lack of diversity for multiplex detection, their clinical application has been restricted to single target analysis. Furthermore, although polymer particles embedded with rare earth metals have been introduced, those particles were relatively large in size (~700 nm) and were designed for mass cytometry of proxy cell [19].

Therefore, smaller nanoparticles doped with various metals were inevitabley needed for multiplex detection in ICP-MS immunoassay. Noticeably, the sandwich-type platform suffered from measurement error particularly caused by particle loss during the treatment without using ratiometric measurement. For the measurement, MNPs doped with interference-free metals were needed because ^{56}Fe and ^{57}Fe ions of MNPs suffer from the heavy molecular interference of $^{40}Ar^{16}O^{+}$ and $^{40}Ar^{16}OH^{+}$ in ICP-MS [20].

In this work, we synthesized Cs-doped multicore magnetic nanoparticles (MMNPs) for magnetic extraction and three different types of metal/dye-doped silica nanoparticles (SNPs) as tagging probes for multiplex detection, i.e., Y/RhBITC (rhodamin B isothiocyanate)-doped SNPs, Cd/RhBITC-doped SNPs, and Au/5(6)-XRITC (X-rhodamine-5-(and-6)-isothiocyanate)-doped SNPs. The particle size were designed to be <~90 nm, which is proper for tagging and extraction of those targets. As targets for multiplex detection, the biomarkers of α-fetoprotein (AFP), neuron specific enolase (NSE), and c-reactive protein (CRP) were selected. AFP is a plasma protein found in the human fetus and protects it from maternal estradiol. A level above 500 ng mL^{-1} can be indicative of hepatocellular carcinoma, germ cell tumors, and metastatic cancers of the liver. NSE represents the dominant enolase-isoenzyme found in neuronal and neuroendocrine tissues, whose levels in serum correlate with a variety of neuron-destructive and neurodegenerative disorders. In the context of a patient with a lung mass, elevated NSE suggests an underlying small cell lung carcinoma. CRP is a ring-shaped pentameric protein found in blood plasma. Since CRP levels rise in response to inflammation, patients with elevated levels are at an increased risk of diabetes, hypertension and cardiovascular disease. The normal concentration levels of those biomarkers in the blood of a healthy human are below 25 ng mL^{-1} for AFP, 15 ng mL^{-1} for NES, and 1 μg mL^{-1} for CRP [21–23].

In this work, the doped Cs of MMNPs were used as an internal standard for ratiometric measurement and the doped Y, Cd, and Au in SNPs were quantified by ICP-MS for the multiplex detection of biomarkers spiked in human serum. For method evaluation, analytical figures of merit including cross reactivity were obtained and the recovery was compared with that of ELISA.

2. Experimental section

2.1. Chemicals

The reagents used for the nanoparticles such as FITC (fluorescein isothiocyanate isomer I, ≥ 90%), RhBITC (rhodamine B isothiocyanate, mixed isomer), 3-APTES (3-aminopropyltriethoxysilane, 99%), TEOS (tetraethyl orthosilicate, 99.999%), THPMP (3-(trihydroxysilyl)propylmethylphosphonate), 50 wt% in H_2O) were purchased from Sigma-Aldrich (St. Louis, MO, USA). XRITC (X-rhodamine-5-(and-6)-isothiocyanate, 94%) was purchased from Life Technologies Inc. (Grand Island, NY, USA). AFP (90005) and its monoclonal antibody (70166) were obtained from Biocheck Inc. (Foster City, CA, USA) and the other antigens and antibodies, including NSE(ab78797, >98%), CRP(ab111647, >90%), and all polyclonal antibodies for immunoreaction, were obtained from Abcam Inc. (Cambridge, UK). CsCl (99.999%), YCl_3(99.99%), $CdCl_2$ (99.99%), $AuCl_3$(≥99.99%), ethanol (EtOH), DMSO (dimethylsulfoxide, ≥ 99.5%), $FeCl_3 \cdot 6H_2O$ (97%), and $FeCl_2 \cdot 4H_2O$ (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Synthesis of metal/dye-doped silica nanoparticles (SNPs)

Four different SNPs, Cs/FITC, Y/RhBITC, Cd/RhBITC, and Au/XRITC-doped silica core, were synthesized. Each metal chloride salt (52 mg CsCl, 9.38 mg YCl_3, 6.3 mg $CdCl_2$, and 11.4 mg $AuCl_3$) were dissolved in 1 mL of de-ionized water (DIW), and their corresponding dyes (6 mg FITC for Cs, 7 mg RhBITC for Y and Cd, and 5 mg XRITC for Au) were dissolved in EtOH and/or DMSO solutions (6 mL of EtOH for FITC, 7 mL of EtOH for RhBITC, and the mixture of 2 mL DMSO and 5 mL EtOH for XRITC). The solutions of each metal and dye core were mixed together and sonicated for 5 min. Then, 3-APTES was added in the ratio of 5 μL per each 1 mg of dye molecule, and the mixtures were stirred for 24 h in the dark.

2.3. Synthesis of Cs-doped multicore magnetic nanoparticles (MMNPs)

MNP cores of iron oxide (Fe_3O_4) were synthesized by alkaline co-precipitation as published in our previous article [24]. In brief, $FeCl_3 \cdot 6H_2O$ (1.35 g) and $FeCl_2 \cdot 4H_2O$ (0.5 g) were dissolved in 25 mL of de-ionized water (DIW). The solution was heated up to 80 °C, and 12.5 mL of NH_4OH was added and reacted for 20 min. The solution was then cooled to 25 °C and the synthesized MNPs were washed using DIW by magnetic separation. For metal doping, Cs-doped SNP cores were prepared by the same procedure used in the previous section. Then, Cs-doped MMNPs were synthesized by a reverse microemulsion method using the MNP and Cs-doped SNP cores. The microemulsion was produced by adding DIW and docusate sodium salt to heptane solution. In this case, 10 mg of the synthesized MNP cores were dispersed in the 860 μL of DIW and added to 48 mL of heptane together with 2.3 g of docusate sodium salt. After stirring for 10 min, 300 μL of the Cs-doped SNP core solution was spiked and reacted for 30 min. Then, 430 μL of TEOS and 260 μL of 26% NH_4OH were added and stirred for 24 h.

In order to modify the surface with amino and phosphonate groups, 55 μL of 3-APTES and THPMP were added and stirred for 24 h. After the reaction, particles were washed three times with acetone, EtOH, and DIW in succession at 4000 rpm. The prepared MMNPs and metal-doped SNPs were stored in PBS buffer solution. The schematic of the summarized synthesis procedures was shown in Fig. 1.

2.4. Immobilization of antibodies and application

For immunoreaction, polyclonal and monoclonal antibodies were immobilized on the MMNPs and SNPs, respectively. The MMNP solutions (4 mL) were dispersed in 20 mL PBS solution, and 1 mL SNP solutions were dispersed in 10 mL PBS solutions. Those solutions were sonicated for 1.5 h prior to use. For immobilization, 200 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (NHS), well-known zero-cross linkers, were dissolved in 5 mL PBS solution. Then, 2 mL of MMNPs and 1 mL of SNPs were spiked and stirred for