HIGHLY SENSITIVE IMMUNOASSAY USING SURFACE-ENHANCED RAMAN SCATTERING OF HOLLOW GOLD NANOSPHERES IN A MICROFLUIDIC CHANNEL

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ABSTRACT

We reports a quick and reproducible surface-enhanced Raman scattering (SERS)-based immunoassay technique using hollow gold nanospheres (HGNs) and magnetic beads. HGNs show strong SERS enhancement effects from individual particles because hot spots can be localized within pinholes in the hollow particle structure. Accordingly, HGNs can be used for highly reproducible immunoanalysis of cancer markers. Magnetic beads were used as supporting substrates for the formation of the immunocomplex. In addition, a microfluidic platform allows the integration of magnetic bars for low-volume immunoassays. A SERS-based sandwich immunoassay has been performed on chip in an automatic manner.

KEYWORDS: Hollow gold nanospheres, Immunoassay, SERS, Microfluidics

INTRODUCTION

This paper reports a quick and reproducible surface-enhanced Raman scattering (SERS)-based immunoassay technique, using hollow gold nanospheres (HGNs) and magnetic beads. Here, HGNs show strong SERS enhancement effects from individual particles because hot spots can be localized on the pinholes in the hollow particle structure [1]. Thus, HGNs can be used for highly reproducible immunoanalysis of cancer markers [2]. Magnetic beads were used as supporting substrates for the formation of the immunocomplex. In addition, a lab-on-a-chip technology, to integrate magnetic bars into microfluidic devices for low-volume immunoassay, has been developed to reduce the consumption of reagents. SERS-based sandwich immunoassay has been performed on chip in an automatic manner [3]. For the validation of our SERS immunoassay, a well-known lung cancer marker, carcinoembryonic antigen (CEA), was used as a target marker

EXPERIMENTAL

Cobalt nanoparticles were synthesized by reducing $CoCl_2$ with $NaBH_4$ under an N_2 purging condition and were used as templates for HGNs. $HAuCl_4$ was added 10 times in 50 μL aliquots. Here, gold atoms were nucleated and grown up to small shells around the cobalt template. When the solution was exposed to ambient conditions by stopping the N_2 purging, the cobalt was completely dissolved and hollow interior was formed. To use these HGNs as SERS-active probes, Raman reporter

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(4,4'-dipyridyl) and antibody were adsorbed onto the surface of the HGNs. Microfluidic devices were fabricated in polydimethylsiloxane (PDMS) using standard soft lithographic techniques. SERS measurements were performed using a Renishaw 2000 Raman microscope system (Renishaw, U.K.). A Melles Griot He-Ne laser operating at λ= 632.8 nm was used as the excitation source with a laser power of approximately 30 mW.

RESULTS AND DISCUSSION

Figure 1 shows the layout and photograph of the multi-splitting microfluidic device for generating gradients. The purpose of this microfluidic network is to generate different concentrations of target antigen samples in a controlled way. Functionalized magnetic beads and HGNs were introduced at the second inlets to make a sandwich immunocomplex. This is captured by the magnetic bars close to the outlet of the microfluidic channel, allowing the SERS signals to be measured. Figure 2 shows the schematic illustration of functional HGNs and magnetic beads for the formation of a CEA sandwich immunocomplex. The average diameters of HGNs and magnetic beads were estimated to be 45 nm and 1 μm, respectively. Figure 3 shows a schematic representation of the experimental protocol for the SERS-based magnetic immunoassay. Figure 4 illustrates SERS spectra of the sandwich immunocomplexes for various concentrations of CEA antigen. It also shows the corresponding ratio of the SERS signal at 1612 cm^{-1} for the logarithmic concentration of CEA. The inset figure shows an excellent linear response in the lower concentration range from 0 to 100 pg/mL.

CONCLUSIONS

Our proposed SERS-based immunoassay technique, with antibody-conjugated HGNs and magnetic beads, has many advantages over previously reported SERS detection methods, such as good reproducibility, low limits of detection and fast assay times. Accordingly, this technique is expected to be a powerful clinical tool for fast and reliable cancer diagnosis.

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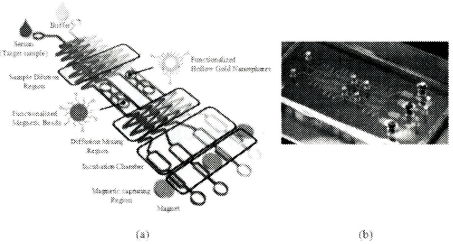

Figure 1. (a)Layout and (b)photograph of PDMS chip for the realization of microfluidic immunoassay.

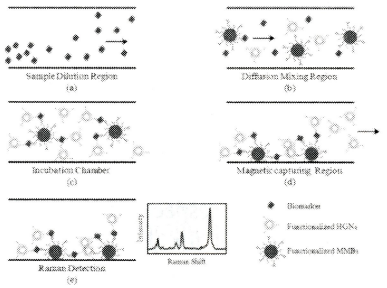

Figure 2. A schematic diagram of the procedure for the on-chip generation of immunocomplexes and signal detection in each parts of the microfluidic channel.

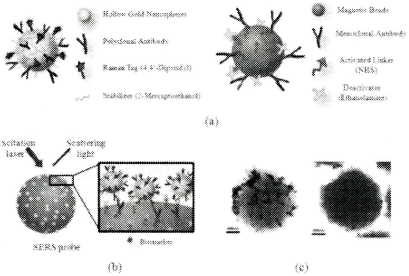

Figure 3. Illustration of SERS immunoassay using HGNs and magnetic beads: (a) probes; conjugation of Raman tag (4,4'-dipyridyl) and polyclonal CEA antibodies onto HGNs (left) and modification of monoclonal CEA antiboies onto magnetic beads (right) (b) formation of the sandwich immunocomplex between antibodyconjugated HGNs and magnetic beads and (c) TEM images of a single magnetic bead with CEA antigens (left) and without CEA antigens(right).

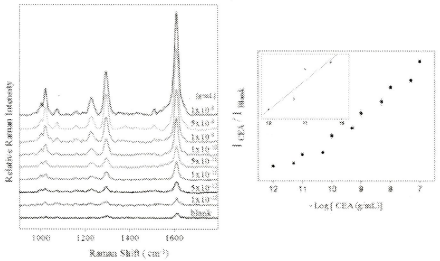

Figure 4. (a) SERS spectra for decreasing concentrations of CEA antigen and (b) corresponding intensity ratio (I_{CEA}/I_{Blank}) of the SERS signal at 1612 cm^{-1} for the logarithmic concentration of CEA. Inset shows a linear relationship in the lower concentration range from 1 to 100 pg/mL (coefficient of determination, R^2=0.93). Error bars indicate standard deviations from four measurements.