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Detection of Hepatitis B virus antigen from human blood: SERS immunoassay in a microfluidic system

Agnieszka Kamińska [a,*], Evelin Witkowska [a], Katarzyna Winkler [a], Igor Dzięcielewski [b], Jan L. Weyher [b], Jacek Waluk [a]

[a] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
[b] Institute of High Pressure Physics, Polish Academy of Sciences, Sokołowska 29/37, 01-142 Warsaw, Poland

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ABSTRACT

A highly sensitive immunoassay utilizing surface-enhanced Raman scattering (SERS) has been developed with a new Raman reporter and a unique SERS-active substrate incorporated into a microfluidic device. An appropriately designed Raman reporter, basic fuchsin (FC), gives strong SERS enhancement and has the ability to bind both the antibody and gold nanostructures. The fuchsin-labeled immuno-Au nanoflowers can form a sandwich structure with the antigen and the antibody immobilized on the SERS-active substrate based on Au–Ag coated GaN. Our experimental results indicate that this SERS-active substrate with its strong surface-enhancement factor, high stability and reproducibility plays a crucial role in improving the efficiency of SERS immunoassay. This SERS assay was applied to the detection of Hepatitis B virus antigen (HBsAg) in human blood plasma. A calibration curve was obtained by plotting the intensity of SERS signal of FC band at 1178 cm^{-1} versus the concentration of antigen. The low detection limit for Hepatitis B virus antigen was estimated to be 0.01IU/mL. The average relative standard deviation (RSD) of this method is less than 10%. This SERS immunoassay gives exact results over a broad linear range, reflecting clinically relevant HBsAg concentrations. It also exhibits high biological specificity for the detection of Hepatitis B virus antigen

1. Introduction

Surface-enhanced Raman spectroscopy (SERS), with its ability of nondestructive, ultrasensitive, reliable and fast detection of samples, has been extensively employed for analyzing complex biological molecules, ranging from DNA (Ke et al., 2005; Sanchez-Cortes et al., 2002) and peptides (Ortiz et al., 2004; Stewart and Fredericks, 1999) to whole proteins (Chowdhury et al., 2006) and cells (Huang et al., 2006). The most notable recent advances in SERS include innovative applications of biomolecular sensors for clinical diagnosis, biochemical, and environmental studies. In particular, a SERS immunoassay, which is based on a specific interaction between an antigen and a complementary antibody is a powerful analytical tool for clinical diagnosis (Driskell et al., 2007, 2005; Kim et al., 2006). Conventional immunoassays include radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). SERS-based immunosensors generally rely on the SERS tags consisting of Raman reporters, active substrates (silver or gold nanostructures), and conjugated antibodies (Grubisha et al., 2003; Han et al., 2008a, 2008b). Typically, the SERS-based immunoassays were carried out according to a standard sandwich protocol of ELISA. For example, an antibody immobilized on a solid substrate reacts with an antigen, which binds to another antibody labeled with peroxidase. If this immunocomplex is subjected to the reaction with *o*-phenylenediamine and hydrogen peroxide, azoaniline is generated, giving a strong SERS signal. Some authors (Dou et al., 1997; Xu et al., 2004) utilized the immunoreaction between immunogold colloids modified by Raman-active probe molecules, e.g. 4-mercaptobenzoic acid, and antigens, which were captured by antibody-assembled chips. Application of SERS in immunosensing included also a successful detection of the thyroid stimulating hormone (THS) (Rohr et al., 1989), monitoring the immunocomplex formation between mouse IgG and goat anti-mouse IgG (Zhang et al., 2008) or detection of membrane-bound enzymes within cells (Hawi et al., 1998). The strength of SERS-based detection lies in the combination of its high sensitivity and facility for multiplexing. Multiple analyses can be performed by using nanoparticles coated with different Raman reporter molecules and by immobilizing mixtures of different antibodies. Additionally, Raman responses are much less susceptible to photobleaching than fluorescence, allowing extended signal averaging in order to lower detection limits (Goluck et al., 2006).

* Corresponding author.

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Previous SERS immunoassays were usually based on extrinsic Raman labels consisting of gold nanoparticles that were coated with co-adsorbed Raman reporter and antibody (Song et al., 2009; Yoon et al., 2010). This strategy has several disadvantages. First, weakly adsorbed antibodies may desorb from one metal particle and re-adsorb on another. This may lead to a spectral signature related to non-specific adsorption and hence hamper multiplex analysis. Second, problems appear with the particles aggregation, which may also be caused by the desorption of antibodies. In order to solve these problems, an alternative approach was investigated in the present study. Our Raman reporter molecule was strategically designed to chemisorb on a thiolate layer on a gold nanoparticle, to provide unique and strong Raman spectrum, and to covalently bind the antibody via a terminal amino group. This group can react with the carboxylic group of a protein activated earlier by EDC/NHS standard procedure to form an amide linkage (EDC=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and NHS=hydroxysulfosuccinimide; see Supplementary material for more details). The added advantages of our method reflect the use of gold-coated GaN substrate as the immune-solid platform. The SERS-active solid substrate plays an important role in the sensitivity of SERS-based immunoassay. It was found that the enhanced electromagnetic field is not only excited around the gold or silver nanoparticles, but is also generated on the SERS-active substrate (Kusnezow and Hoheisel, 2003; Song et al., 2009). In the past, most substrates in SERS-based immunoassays were prepared without SERS activity. Among them, glass slides were used most widely as solid SERS supports (Zhang et al., 2007; Cao et al., 2003). We have developed a novel SERS-active substrates based on Au or Au–Ag coated GaN with bunched nanopillars (Kamińska et al., 2011; Weyher et al., 2012). The resultant SERS platforms exhibit very strong surface-enhancement factor (up to 1×10^7), high stability (up to three months under ambient conditions) and high reproducibility, which could be used in the design of efficient SERS-active platforms for analytical applications. Moreover, the incorporation of our solid SERS platform into a microfluidic device offers a significantly larger active surface for immune reactions and hence improved performance of the immunoassay.

Finally, we combined the advantages of our unique SERS-active substrate based on Au–Ag coated GaN with those of the labeling method and developed a novel SERS immunoassay to detect Hepatitis B virus antigen (HBsAg) in human blood or blood plasma within a microfluidic system. Hepatitis B virus (HBV) infection is a very common cause of chronic liver disease worldwide and the HBsAg is most frequently used to screen for the presence of this infection. HbsAg is a complex particle, about 20 nm in diameter,

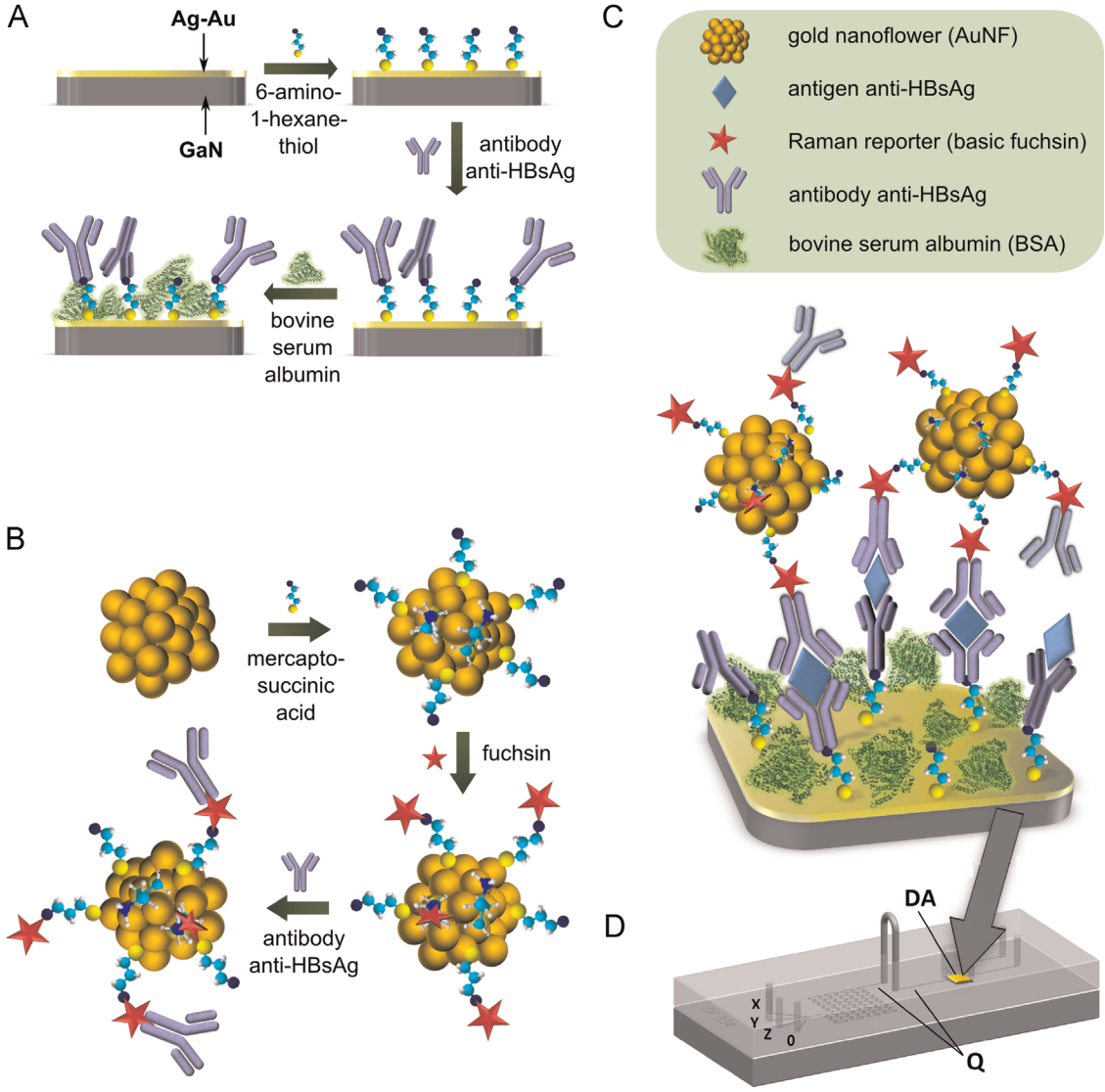

Fig. 1. Sequential steps for the formation of the SERS-based. immunoassay. (A) The capturing substrate preparation, (B) the Raman reporter-labeled immuno-Au nanoflowers synthesis, and (C) SERS detection of the sandwich interactions, (D) schematic illustration showing the integration of a microfluidic device with the SERS-active substrate based on Au–Ag coated GaN surface. DA, detection area chamber with GaN/Au–Ag SERS substrate.