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Heteroassembled gold nanoparticles with sandwich-immunoassay LSPR chip format for rapid and sensitive detection of hepatitis B virus surface antigen (HBsAg)

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ABSTRACT

This study aimed to develop a more sensitive method for the detection of hepatitis B surface antigen (HBsAg) using heteroassembled gold nanoparticles (AuNPs). A single layered localized surface plasmon resonance (LSPR) chip format was developed with antigen-antibody reaction-based detection symmetry using AuNPs, which detected HBsAg at 10 pg/mL. To further improve the detection limit, a modified detection format was fabricated by fixing a secondary antibody (to form a heteroassembled sandwich format) to the AuNP monolayer, which enhanced the detection sensitivity by about 100 times. The developed heteroassembled AuNPs sandwich-immunoassay LSPR chip format was able to detect as little as 100 fg/mL of HBsAg within 10–15 min. In addition, the heteroassembled AuNPs sandwich-immunoassay LSPR chip format did not show any non-specific binding to other tested antigens, including alpha fetoprotein (AFP), C-reactive protein (CRP), and prostate-specific antigen (PSA). These findings confirm that the proposed detection strategy of heteroassembled AuNPs sandwich-immunoassay LSPR chip format may provide a new platform for early diagnosis of various human diseases.

1. Introduction

Hepatitis B virus (HBV), a member of the Hepadnaviridae family, is the causative agent of hepatitis B. Hepatitis B virus can cause chronic hepatitis, eventually leading to liver failure, cirrhosis, and hepatocellular carcinoma (HCC) (Yildiz et al., 2015). Thus, early diagnosis of HBV infection is necessary to allow rapid treatment and reduce the resulting damage. Hepatitis B surface antigen (HBsAg) is the first indicator and an important biomarker for the diagnosis of HBV infection (Kao, 2008). The cut-off HBsAg concentration for the diagnosis of HBV infection is considered to be in the range of 0.07–0.12 ng/mL (Mukherjee et al., 2010).

A number of diagnostic strategies based on immunogenic assays have been developed for the detection of HBsAg. These include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), chemiluminescence immunoassay (Ehsani et al., 2017), quartz crystal microbalance (QCM) immunoassay (Akter et al., 2015), impedimetric immunoassay (Wiederoder et al., 2016), and electrochemistry immunoassay (Ranjani et al., 2015). However, these detection strategies require extensive efforts by expert users and have variability in interpreting the results. In addition, several advanced molecular PCR-based detection methods have been found to have good sensitivity, but they are not easily portable, are time consuming, and are cost ineffective. To improve field usability, researchers have focused on lateral flow immunochromatographic strip assays to detect HBsAg (Xia et al., 2009). Although these assays are quick and easy to use in the field, they have low detection sensitivity and poor stability.

In recent years, there have been revolutionary developments in nanotechnology applications for biosensing. Various bio-diagnostic nanoparticle-based sensing platforms have been developed for

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detecting low concentrations of analytes with high affinity and specificity and signal transduction into quantifiable information (Hong et al., 2012). Previously, researchers identified some drawbacks in plasmon resonance-based detection, such as non-specific absorption and nanoparticle aggregation, which may cause desorption of bio-receptors such as aptamers or antibodies (Kamińska et al., 2015). Despite these drawbacks, localized surface plasmon resonance (LSPR) sensing assays have shown promise as sensitive diagnostic techniques (Haes and Duyne, 2004). The LSPR sensing technique is based on the surface plasmon resonance phenomenon in which metal electrons are coherently oscillated in the electromagnetic field during surface conduction (Miller and Lazarides, 2005). It has shown a high detection sensitivity due to the sensitive change of the local dielectric environment on the metal nanostructure surface (Miller and Lazarides, 2005). In this context, several types of metal-based (europium, gold, silver, and copper) and non-metal-based (graphene, silica, and carbon nanotubes etc.) nanoparticles have been used in biosensing strategies for a variety of biological analytes (Bansal et al., 2014; Paterson et al., 2017; Xia et al., 2009). It is well known that gold nanoparticles have a larger light absorption and scattering cross section in the surface plasmon resonance wavelength regions (Wang et al., 2010). Therefore, we aimed to construct a portable LSPR sensing chip for HBsAg detection that would meet all the criteria for its use in real-time clinical applications.

In the present study, a diagnostic platform was assembled to achieve an amplified LSPR signal on approaching anti-HBsAg conjugation with heteroassembled AuNPs in a sandwich-immunoassay LSPR chip format. LSPR signals were increased owing to the repulsive force due to the surface charge of the proximal gold nanoparticles, resulting in detection of low concentrations of HBsAg with high sensitivity. Further, to confirm the superior ability of the developed LSPR chip, a comparative study was performed with commercial and previously developed lateral flow assay strips.

2. Experimental section

2.1. Antibody and antigens

Anti-HBsAg antibody and HBsAg were purchased from Boreda Biotech (Korea). Alpha fetoprotein (AFP), C-reactive protein (CRP), and prostate specific antigen (PSA) were purchased from Boreda Pvt. (Korea), Hytest LTD (Turku, Finland), and Fitzgerald Industries International (Acron, MA, USA), respectively. Other chemicals and solvents used in this study are reported in the Supporting materials under sub-heading 2.1.

2.2. Experimental design for AuNPs based LSPR sensing chip

AuNPs were synthesized using the previously described gold nanoseed growing method (Bastús et al., 2011). The surface of glass slides (5 cm × 0.8 cm [length × width]) were fabricated with synthesized AuNPs by the treatment of 0.5% APTES solution (in distilled water). After fabricating LSPR sensing chip, conjugation of anti-HBsAg antibody in fabricated glass substrate was optimized with specified antibody concentrations. The details of the AuNPs synthesis procedure, antibody conjugation steps, characterization and fabrication of LSPR sensing chips can be found in the Supporting materials under sub-heading 2.2, 2.3, 2.4 and 2.5, respectively.

2.3. Detection assay format

The developed LSPR chip format consisted of a self-assembled device with a glass substrate fabricated with AuNPs and conjugated with an anti-HBsAg antibody (Fig. 1A). After inoculation of a target antigen (HBsAg) solution (analyte) onto the prepared sensing chip and a 10-min incubation, the LSPR signals were detected by measuring the absorbance spectra at 400–700 nm. To enhance detection sensitivity and specificity, we designed a sandwich immunoassay format of LSPR chip using a second layer of AuNPs conjugated with anti-HBsAg antibody at a concentration of 10 μg/mL (immunocolloid AuNPs) to obtain heteroassembled AuNPs sandwich-immunoassay chip format (Fig. 1B). Test samples were compared to a blank chip without HBsAg addition.

2.4. Detection sensitivity of the developed LSPR chip formats

In order to achieve detection sensitivity in a single and heteroassembled AuNPs sandwich assay LSPR chip formats, we assessed LSPR signal detection at different concentrations of HBsAg (1 pg/mL–10 μg/mL). In brief, the LSPR chip was dipped in various concentrations of HBsAg followed by 10 min of incubation at room temperature to allow interactions between the antibody-conjugated AuNP and HBsAg. Detection sensitivity was then analyzed by measuring the LSPR peak shifts. All experiments were repeated at least three times.

2.5. Specificity of the detection test

The developed heteroassembled AuNPs sandwich-immunoassay LSPR chip was tested for determining its specificity and cross-reactivity in the presence of other antigens, including AFP, CRP, and PSA, as well as in a complex mixture of antigens (AFP + CRP + PSA + HBsAg) at a concentration of 1 μg/mL. These experiments were performed in order to observe the effect of background antigens on the detection of HBsAg.

2.6. Clinical applicability of the device in human serum sample

Commercially procured human serum was spiked with surface HBsAg at various concentrations (100 fg/mL–1 μg/mL) and used as a real sample to test the detection performance of the developed LSPR sensing chips. Detailed methodology can be found in the Supporting materials under sub-heading 2.6.

3. Results and discussion

3.1. Synthesis, characterization and conjugation of AuNPs with anti-HBsAg antibody

Based on optimal parameters, three different sizes (15, 30, and 50 nm) of spherical AuNPs (Fig. S1A, B, and C) were prepared and conjugated with anti-HBsAg antibody. Absorbance spectra of both conjugated and unconjugated AuNPs, measured in the wavelength range from 700 to 400 nm were compared (Fig. S1D, E, and F). The mean hydrodynamic particle size, zeta potential, and PDI values of antibody-conjugated and unconjugated AuNPs are presented in Table S1. The detailed results and discussion for synthesis, characterization and conjugation of anti-HBsAg antibody with AuNPs can be found in the Supporting materials under sub-heading 3.1.

3.2. Optimized conditions for immobilization of anti-HBsAg on the fabricated LSPR chip

Different concentrations of anti-HBsAg antibody (1 ng/mL–1 mg/mL) were analyzed for the detection performance on the LSPR chip fabricated with AuNPs (Fig. S2). As a result, 100 μg/mL of anti-HBsAg was determined to be the optimum to functionalize the LSPR biosensor chip. Detailed explanatory results and discussion can be found in the Supporting materials under sub-heading 3.2.

3.3. Detection sensitivity of LSPR chip with single and heteroassembled AuNPs in a sandwich format

In the current study, the LSPR chip was composed of AuNPs, anti-HBsAg antibodies, and detectable surface antigen. These chips were tested for detection performance against surface anti-HBsAg antibody.