Research Article

Gold Nanoparticles Modified a Multimode Clad-Free Fiber for Ultrasensitive Detection of Bovine Serum Albumin

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Gold nanoparticles (Au NPs) were almost chosen as the first option for biological and biosensor applications due to their enhancement and their outstanding properties. The combining of optical fiber with localized surface plasmon resonance (LSPR) for forming a biosensor is widely used in diagnosis. In this work, we report a fiber optical biosensor based on LSPR of Au NPs for the detection of bovine serum albumin (BSA) protein. BSA was functionalized on Au NPs immobilized fiber optic sensing head (length of 1 cm) via methanesulfonic acid (MSA) by carboxylic binding. It is the binding between the analytes with the surface-modified Au NPs that caused refractive index changes in the sensing medium led to changes in optical power at the output of the sensor. The detection limit of the LSPR fiber biosensor was found to be 0.18 ng/mL for the BSA detection with the low coefficient of variation (CV) at under 1%. We have demonstrated the effectiveness of combining multimode fiber with Au NPs to generate the biosensor as the label-free sensor that can be a feasible tool for highly sensitive, rapid response time, stable, and miniaturized point-of-care analytical systems.

1. Introduction

Gold nanoparticles (Au NPs) with unique properties such as excellent compatibility, intense light scattering/absorption, high surface area to volume ratios, highly selective interoperability through electrostatic interaction, stable structure, and nontoxic have become the first choice among plasmonic nanoparticles for biological and biosensor applications [1–3]. Au NPs also have a special phenome that is localized surface plasmon resonance (LSPR), which was widely studied recently in sensing platforms due to its great advantages [4–6]. It provides compact, label-free, highly sensitive, and stable

biosensing for the detection of biological molecules [7, 8]. LSPR is a phenomenon in metallic nanostructures related to the resonance of free-electron waves in a metal. The incident light could be in resonance with the oscillations of the surface electron at an excitation frequency, resulting in the collective oscillation of the surface plasmons, and it is called an LSPR mode [9].

It is due to the unique optical properties and surface chemistries that Au NPs were used as the promising nanomaterial in numerous different types of sensors such as surface-enhanced Raman scattering (SERS), fluorescence, electrochemical, and fiber optical-based LSPR sensor.

Therein, the combining of optical fiber with the LSPR phenomenon helps to enhance the light-matter interaction that provided a sensor type with enhanced sensitivity, fast response, higher stability, more affordable, compact size, and lower limit of detect sensor than that others [10, 11]. The basic principle of this sensor relies on the alter reflective index of ambient dielectrics around the sensing region and does not require the labeling of the target molecules.

In this work, Au NPs were synthesized via the seed-mediated growth method for the fabrication of the LSPR-based optical biosensor for the detection of a standard protein in biosensor experiments that is bovine serum albumin (BSA). Mercaptosuccinic acid (MSA) with carboxylic acid ($-\text{COOH}$) groups was modified on the sensing surface via Au NP thiol bond and was utilized for the immobilization of BSA. The properties of Au NPs were tracked through the spectroscopic measurements including UV-visible spectroscopy, powder X-ray diffraction (PXRD), and field emission scanning electron microscope (FESEM). Moreover, Fourier-transform infrared spectroscopy (FTIR) and water contact angles (WCAs) were used to confirm the presence of surface functional groups. We achieved a low limit of detection (LOD) of 0.18 ng/mL for BSA detection. This result in this study can be compared to be 5284 times better than Peng et al.'s work [12] about optical biosensors based on surface plasmon polaritons for detecting BSA and 20 times better than that Tran et al.'s [13] about optical fiber biochemical sensors. This result proved the effective combining between the optical properties of Au NPs and optical devices, unfolded potentials in biological analysis, and biosensor application.

2. Experimental Section

2.1. Materials and Reagents. Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.9%), (3-aminopropyl)triethoxysilane (APTES, 99%), Mercaptosuccinic acid (MSA, 97%), sodium citrate tribasic dihydrate ($\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$, Na₃Ctr, 99%), phosphate-buffered saline (PBS), and bovine serum albumin (BSA) were all supplied by Sigma-Aldrich Co., MO, USA. Ethanol ($\text{C}_2\text{H}_5\text{OH}$, 99.8%) was obtained from Fisher Ltd. (UK), and glycerol ($\text{C}_3\text{H}_8\text{O}_3$, 99%) was provided by Duksan Pure Chemicals Co. (Ltd., Korea). Sodium hydroxide (NaOH , 96%) was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (China).

2.2. Synthesis of Gold Nanoparticles. Gold nanoparticles were synthesized by the seed-mediated growth method utilizing citrate reduction of gold(III) chloride trihydrate. Deionized (DI) water was used for all preparations. The seed nanoparticles were made by adding Na₃Ctr rapidly into a round bottom storage flask containing the mixture of 200 μL of NaOH 1 M and 100 mL of HAuCl_4 1 mM at 100°C under vigorous stirring. The solution was stirred stably for 15 minutes and stored at 4°C. Then, the resulting Au seeds were grown by adding 3.377 mL seed into 36.22 mL DI water, 0.176 mL Na₃Ctr, and 0.227 mL of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ 1 mM. The reaction was conducted in an ultrasonic bath at RT for

40 minutes. The final product was preserved in glass vials and stored at 4°C for further use.

2.3. Surface Modification of Fiber Core for BSA Detection. Multimode optical fibers have been used for the construction of this type of sensor for a number of reasons as follows. It is suitable for a wide variety of luminescent sources so it can be expanded for more applications. The larger core makes it easier to denature the surface for sensor application. It is easier to adjust and introduce light to the multimode fiber so it is easier to experiment.

The clad-free fiber (Multimode, NA 0.37, JFTLH-Polymicro Technologies) with the length of 1 cm core sensing has been fabricated by removing part middle of the plastic buffer layer with a soldering machine (ATC-2450-III, ARIM, Korea), and the cladding layer with a mixture of acetone and ethanol (3 : 1). After removing, the silica (SiO_2) surface of the sensor head was modified with Au NPs for the LSPR sensor; then, the carboxylic groups were created on it for BSA detection as shown in Figure 1. Briefly, the fiber core surface was silanized using 3% APTES after the generation of the hydroxyl groups ($-\text{OH}$) on it by an oxygen plasma machine (CUTE-1MPR, Femto Science Inc., Korea) for 2 min. Subsequently, Au NPs were immobilized on the amine-modified sample via the strong electrostatic force. The immersing time for Au NPs to be coated on the surface was investigated (4, 8, 12, and 16 hr). Finally, the clad-free fiber was carboxyl-functionalized using MSA acid solution of 0.1 mM in ethanol for 16 hr at RT, then washed with methanol several times.

2.4. Optical Setup for Biosensor Measurements. Microfluidic fabrication for real-time optical biosensor measurement was proposed and implemented. Briefly, this device is constructed from the bonding PDMS (polydimethylsiloxane, Sylgard 184, Dow Corning Co., USA) mold and clean glass together as soon as they are treated by an oxidation plasma system with the installation of a clad-free fiber inside. The PDMS mold with two ports (inlet/outlet, a diameter of 1 mm) and a straight-shaped flow channel are generated from pouring the mixture of cross-linker curing agent and silicone elastomer base (v/v, a ratio of 1 : 10) into a mold and dried at 70°C during 30 minutes in an oven.

Figure 2 shows the optical measurement system including a 5 mW He-Ne laser (632.8 nm, LASOS LGK 7628), a coupling system (NA of 0.4), an aperture collimator mount (AD9.5F), a digital handheld optical power (PM 100D, Thorlabs, Newton, NJ, USA), and the peristaltic pump (Eleya, SMP-21, Japan) to inject the analyte solution into the channel of microfluidic. Two ports for injecting liquids into the channel were made from the plastic tubing (Eleya, Japan) with inner diameter : outer diameter = 1.15 : 3.2 mm.

2.5. Measurement Techniques. The gold nanoparticles were characterized utilizing UV-visible spectroscopy (V-730 visible/NIR, JASCO, Tokyo, Japan), powder X-ray diffraction (PXRD, Bruker D8 Advance diffractometer, $\lambda = 1.54178 \text{ \AA}$), and field emission scanning electron microscope (FESEM, Hitachi S4800, USA). The presence of the surface functional

FIGURE 1: The illustrations of the surface modification steps for BSA detection.

FIGURE 2: Diagram of the optical measurement system.

groups such as hydroxyl, carboxyl, and amino was investigated by the Fourier transform infrared spectroscopy (FTIR, Bruker Vertex 70, Germany) and water contact angles (WCAs, Phoenix 300, Surface Electro-Optics).

3. Results and Discussion

Au NPs were prepared by the seed-mediated growth method with the reduction of HAuCl_4 utilizing Na_3Cit as a reducing agent. As soon as the synthesis of Au NPs is completed, the change in wavelength value and the color between Au nanoseed and Au NP solution was observed, shown in Figure 3. Briefly, a wine red color was obtained with the LSPR peak at 522.6 nm for the Au nanoseed solution. There are some disadvantages to the seed solution, such as uneven seed size and agglutination between seeds. The use of the intermediate particle development method helps to control the properties of the gold nanoparticles such as absorption size and wavelength, in addition to helping to produce single dispersed Au NPs. As the resulting Au seeds were grown, a slight redshift of the maximum absorbance peak from 522.6 nm to 524.5 nm with the change color to pink was recorded. The Au NPs formation with LSPR of 524.5 nm and the color of solution (Figure 3) implied the appearance of the spherical Au NPs, which is directed in this experiment due to the usefulness of them in bioapplications [14].

FIGURE 3: Wavelength shift in the UV-vis spectrum after the growth of Au NPs from Au nanoseed.

The proof of the presence of the characteristic functional group vibrations after the surface modification steps was recorded via FTIR spectroscopy and WCAs goniometry, as shown in Figure 4. In the FTIR spectrum (Figure 4(a)), the adsorption band at 450, 687, and 1140 cm^{-1} is attributed to Si-O-Si bending (δ), symmetric O-Si-O stretching (ν), and asymmetric Si-O-Si stretching (ν_{as}) vibrational, respectively [15]. The broadening in a band at 3370 to 3500 cm^{-1}

FIGURE 4: (a) FTIR spectra and (b) WCA goniometry of the modified silica surfaces.

FIGURE 5: (a) XRD spectra and (b) FESEM image of the sample at the various gold immersing times (4, 8, 12, and 16 hr).

indicated the signal of -OH groups of the Si-OH stretching vibration and retained water [16]. When modified with APTES, the IR spectrum was changed with the appearance of two peaks that is the N-H stretching and N-H bending vibrations at 3350 and 1570 cm⁻¹ [17]. In the spectrum of Au NP-MSA treatment, the amine vibration disappeared and the emergence of new bands at 1603 and 1340 cm⁻¹ is assigned to asymmetric C=O stretching and the C-O stretching vibration, respectively [18]. In the results of WCA measurement (Figure 4(b)), the wetting behavior of the modified surfaces (the hydrophilic or hydrophobic) was investigated. Briefly, oxygen plasma-treated pristine glass indicated hydrophilicity due to a decrease from the angle of 43.22° ± 0.42 to 11.71° ± 2.94. After APTES functionalization, the wetting angle was increased to 74.23° ± 1.11, imply-

ing the hydrophobic layer on the surface. Then, Au NP-coated glass was determined to be more hydrophobic than the amine-modified surface with an angle of 75.13° ± 6.18. After modification of Au NP-treated surface with MSA acid, the contact angle was increased sharply to 87.74° ± 0.41, shown to turn super hydrophobic of the surface. The presence of the characteristic functional group vibrations in FTIR results and the surface wetting change in WCAs has indicated that -OH, -NH₂, and -COOH groups were successfully formed on the wafer surface.

The properties of Au NPs including the shape, size, crystal structure were investigated via XRD spectra and FESEM image, as shown in Figure 5. The results show that the gold crystallines on the sample surface have the face-centered cubic (fcc) structure with (111), (200), (220), and (311)

FIGURE 6: Time-dependent return light intensity via the optical fiber with the change of BSA concentration (from 0 to 0.1 $\mu\text{g/mL}$).

planes corresponding to 2-theta values of 38.2, 44.4, 64.5, and 77.4° at the different immersing time (4, 8, 12 and 16 hr) (Figure 5(a)) [19]. The (111) plane is the strongest intensity peak indicated that it is the preferred development orientation of Au NPs on the substrate. Furthermore, the shape of Au NPs was displayed as monodispersity spherical nanoparticles with an average diameter of $32\text{ nm} \pm 3$ (Figure 5(b)). Increased immersion time led to an increase in particle count and the dispersion of Au NPs on the surface. According to the investigated results in Figures 5, 12 hr is considered as the appropriate candidate for use in subsequent experiments.

In order to evaluate the optical fiber-based LSPR sensor's performance, BSA was prepared in a list of various concentrations from 0 to 0.1 $\mu\text{g/mL}$ in PBS buffer solution. The BSA solution was injected into the channel of microfluidic where the sensor head was contained. It was the addition of the analyte molecule that caused a change of refractive index (RI) of the sensor medium, which led to an increase or decrease in the light intensity at the output. The basic principle of this sensor is based on the refractive index change of the sensor medium, so it can detect different proteins with different refractive indexes and give different signals. BSA molecules were easily immobilized on the Au NP-covered sensing surface via COOH groups. The refractive index (RI) was altered in the direction of increase by the increasing of BSA concentration, proved in the work of Tran et al. [13]. To determine the LOD of our optical sensor, we measured and recorded the transmittance light in output at various RI of the ambient medium with fixed input light value. The results are presented in Figure 6 showing the sequential increase response of the sensor as the concentration of BSA was increased. The average signal intensity with the error bar at each concentration was recorded for 1 min and was repeated at least 3 times. Then, the coefficient of variation (CV) was estimated to be $<1\%$, indicating the high reproducibility. This sensor was rated highly sensitive due to the signal that varies significantly and immediately as analyte concen-

TABLE 1: The performance of the sensor for detecting BSA.

Biosensor methods	Detection limit	Ref.
ELISA	0.38 ng/mL	[20]
Vis-IR spectral	0.05 mg/mL	[21]
Reverse-phase high performance liquid chromatography (RP-HPLC)	0.11 $\mu\text{g/mL}$	[22]
Two-dimensional transition metal dichalcogenides assisted optical fiber SPR biosensor	0.45 $\mu\text{g/mL}$	[23]
SPR sensor probe without optical fibers	50 ng/mL	[24]
<i>This study</i>	0.18 ng/mL	

tration changes. The LOD value (ng/mL) of this sensor was calculated as 0.18. This LOD is comparable to that of other sensors as shown in Table 1 indicating the high efficiency of the fiber optical LSPR biosensor. Our sensor therefore with this performance can be recognized as a feasible tool to be applied to biosensing.

4. Conclusion

In this article, an optical real-time measurement system and a biosensor device that contain the Au NP-coated fiber sensor head have been fabricated for sensor application. Au NPs have been synthesized by the seed-mediated growth method with the investigation of seed development through ultrasonic energy along with time change to find out the optimization in the properties of nanoparticles. Moreover, the process of surface denaturation of the sensor head of fiber optics with the modification of hydroxyl, amine, Au NPs, and carboxylic, respectively, for the analyte detection has been proposed. The combination of optical properties, X-ray diffraction technique, UV-vis spectroscopy, IR, and FESEM was used to follow the formation of crystal growth, absorption plasmon peak, sizes, and shapes of Au NPs. The high LOD of the LSPR sensor achieved 0.18 ng/mL for detection of BSA protein with remarkably high reproducibility. This research presented an in situ biosensor with highly sensitive in a compact format suited to the point-of-care testing.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Vu Thi Huong and Nguyen Tran Truc Phuong contributed equally to this work.

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