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Contributed Paper

Gold-nanoparticle-based Fiber Optic Sensor for Sensing the Refractive Index of Environmental Solutions

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ABSTRACT

Here an optical fiber modified with gold nanoparticles was successfully fabricated to sense the refractive index of a chemical solution surrounding its surface, with the potential of utilizing this sensor as a biosensing device. Gold nanoparticles, with an average diameter ~20 nm, were synthesized via the citrate reduction method and used to functionalize the glass core of an optical fiber. The sensing principle, which is based on localized surface plasmon resonance, requires the metal gold nanoparticles to be exposed to both the incident and absorbing lights, with the potential detection capabilities determined from the observed light intensity measurement. The optical fiber was uncladded at its center to expose the glass core fiber, and gold nanoparticles were immobilized on the exposed surface using a silane coupling agent. The sensitivity of the gold-nanoparticle-modified optical fibers in measuring the refractive index changes of a solution was compared for unclad lengths ranging between 1 and 2 cm. The attenuation of light depended on both the refractive index of solution and the length of the unclad fiber. The results showed that the fiber sensitivity increased as the unclad length increased due to greater amount of gold on the surface. Antibody-A, which has a specific binding to antigen-A, was then covalently linked to the fiber surface via an amide bond to demonstrate the potential bio-sensing platform of this sensor. The antibody-A functionalized optical fiber was used to detect the red blood cell samples in groups A, B, and O, where it effectively detected both the specific and non-specific binding signals. This fiber optic biosensor therefore provides a low-cost and simple fabrication setup that has potential field applications. Moreover, this setup could potentially be applied to detect other types of whole cell samples.

Keywords: fiber optic, gold nanoparticle, refractive index sensor, optical sensor

1. INTRODUCTION

Biosensors have recently been receiving increased attention due to their potential applications in environmental detection and clinical diagnostic research [1, 2]. The biosensor consists of two main parts, the biological sensing element and the transducer that converts the biological interaction into a readable signal. The use of the label-free biosensor technique has the advantage of not requiring fluorescent molecules for the analyte detection. The surface plasmon resonance (SPR) technique is a well-known label-free technique for biomolecular detection. SPR is the phenomenon where the frequency of an incident light that propagates to the metal-dielectric interface resonates with the oscillating electrons on the metal surface [3]. SPR is an optical-based technique that senses the change in the refractive index of the solution near the metal surface. The key advantages of an SPR sensor are its high sensitivity, fast response time, real-time monitoring capability, and label-free approach, as mentioned above. However, SPR sensors are still difficult to manufacture, because the setup is bulky, requiring a prism and also a gold film on the glass surface.

The localized surface plasmon resonance (LSPR) biosensor is another tool that is employed to detect biomolecules. The principle is based on the SPR phenomenon, which occurs in the metal nanoparticles instead of the bulk metallic film. Here the nanoscale size of the nanoparticles is smaller than the wavelength of the light source, which can therefore be excited by the light source, and the resonance frequency of the nanoparticles is dependent on the refractive index of nanoparticle surface interface [4, 5]. These fiber-optic-based biosensors can respond to the interaction between the analyte and the immobilizing ligand due to the generation of an evanescent field near the nanoparticle surface as the light propagates through the optical fiber. The evanescent field excites the conductive electrons of the nanoparticle, which results in the increased absorption and scattering of the light source [6].

The advantages of the LSPR sensor are its small size and its simple fabrication. Here we perform the LSPR biosensor in a transmission-based fiber-optic sensor by using a gold-coated optical fiber with different unclad lengths. The simple setup and sensitivity of the gold-coated optical fibers in different refractive index solutions is examined. The ability of the sensor to detect biomolecules is confirmed by studying the interaction between immobilized antibody-A and antigen-A on the red blood cell surface for analyte detection.

2. MATERIALS AND METHODS

2.1 Reagents and Materials

Gold (III) chloride hydrate ($HAuCl_4$), sodium citrate tribasic dehydrate, (3-Aminopropyl)triethoxysilane (APTES), 11-Mercaptoundecanoic acid (11-MUA), (3-Dimethylaminopropyl)-N2-ethylcarbodiimide hydrochloride (EDC), and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. A multimode optical fiber (model FT400 UMT) was purchased from Thorlabs, USA, with core and cladding diameters of 400 and 430 μm, respectively. The phosphate buffer saline (PBS) tablets that were used were from PT Biogen Scientific. The antibody-A and red blood cell samples were purchased from the Thai Red Cross Society, Thailand. All solutions were prepared using distilled (DI) water.

2.2 Preparation of the Gold Nanoparticles

The gold solution was synthesized following the procedure outlined in a previous report [7]. A 100-mL solution of 0.01% $HAuCl_4$ was heated to a boil in a round bottle flask, and 1.5 mL of 1% sodium citrate was then added to the solution. The solution turned blue, and then changed to a red-violet color. The solution continued to boil for another 10 min, and was then removed from the heat. The solution was stirred for 15 min, and the gold solution was stored in a refrigerator until use. The absorbance spectrum of the gold solution was measured with a mini-spectrometer (TG series, Hamamatsu).

2.3 Gold Nanoparticle on the Modified Optical Fiber Surface

The 20-cm-long optical fibers were uncladded at their centers, with lengths of 1, 1.5, and 2 cm chosen for this analysis. The unclad region was abraded and cleaned in acetone for 20 min, followed by a DI water rinse and drying with N_2 gas. The 5% APTES solution in ethanol was prepared, and the glass core fiber was incubated in the solution for 2 h. The fiber was then rinsed with ethanol and DI water ten times before the next incubation in the synthesized gold nanoparticle solution overnight. After that, the glass core of the fiber was rinsed with DI water to remove any excess gold nanoparticles from the surface, followed by drying with N_2 gas and storage in desiccators until it was used. The depositions of gold nanoparticles on the unclad optical fiber were examined by field-emission scanning electron microscopy (FE-SEM) using a JEOL JSM-7800F Prime microscope. An energy dispersive X-ray analysis (EDS) was performed on the gold-coated optical fiber to confirm the presence of gold on the surface.

2.4 Refractive Index Measurements

The 0.5-10% glycerol solutions were prepared such that the solutions had different refractive index values (1.334 to 1.350). The setup for the optical fiber experiments was constructed with the white light source (Type-A GCS, Mightex) connected to the mini-spectrometer using the gold-coated optical fiber in the middle. The sample measurements were made in a small acrylic chamber, with the chamber placed to cover the sensing region of the gold-coated fiber that could then be moved during the alteration of the samples. The series of glycerol solutions were added into the chamber, each possessing a volume of 150 µL, with the light intensity spectrum then collected for each solution. The transmission intensity decreased as the refractive index increased. The normalized intensity was calculated from the sample signal (I_s) divided by the reference signal (I_r), which was then evaluated to obtain the refractive index sensitivity. The signal was converted to % transmittance by $(I_s/I_r) \times 100\%$ to assess the biosensor platform.

2.5 Functionalization of the Gold Nanoparticle-optical Fiber with then Antibody Probe

The sensing region of the gold-coated optical fiber was immersed in 10 mM of 11-MUA in methanol solution for 18 h. The self-assembled monolayer of 11-MUA was functionalized on the gold-coated surface, which provided the carboxylic as the terminate functional group. The excess 11-MUA was washed with methanol and then rinsed with DI water ten times. An EDC/NHS solution at 0.2/0.05 M was used to incubate the sensing region for 10 min, followed by the PBS buffer, to activate the carboxylic group. Then, 150 µL of antibody-A in sodium acetate (pH = 5) was added

into the chamber and left until the reaction of antibody linked onto the surface for 15 min. The PBS buffer was then used to wash away any loosely bound antibodies. The rest of the activated carboxylic group on the surface was blocked with ethanolamine solution for 10 min, followed by another PBS buffer wash. The gold-coat optical fiber functionalized with the antibody-A probe was then ready to use for measuring the specific antigen.

2.6 Detection of Antigens on the Red Blood Cell Surface

The modified optical fiber with gold nanoparticles was used to measure the antigens on the red blood cell surface to assess the capability of the optical fiber for biomolecular detection. The 5% red blood cell sample solutions of groups A, B, and O were tested with the immobilized antibody-A on the gold-coated optical fiber. A red blood cell sample volume of 150 mL was added into the small chamber, with the unclad region of the fiber positioned inside. This chamber possessed the flexibility to move the fiber in and out, as well as change the sample solution, as described in section 2.4. The spectrum intensity of the PBS buffer was collected before and after immersing the sensing region into the red blood cell sample. The specific and non-specific signals were compared to the change in % transmittance (Δ%T) of the PBS buffer before and after immersing the sensor in the red blood cell sample.

3. RESULTS AND DISCUSSION

3.1 Gold Nanoparticle on the Modified Optical Fiber

The ultraviolet-visible (UV-Vis) absorption of the synthesized gold solution was detected using a mini-spectrometer, with the absorbance spectrum shown in Figure 1. The gold colloid had a maximum absorption (λ_{max}) peak at ~520 nm. This absorbance peak can be calculated to the size of a spherical gold nanoparticle of ~23 nm, where we followed the calculation method employed in a previous report [8] that analyzed the sizes of uncoated spherical gold nanoparticles in water using UV-Vis spectra.

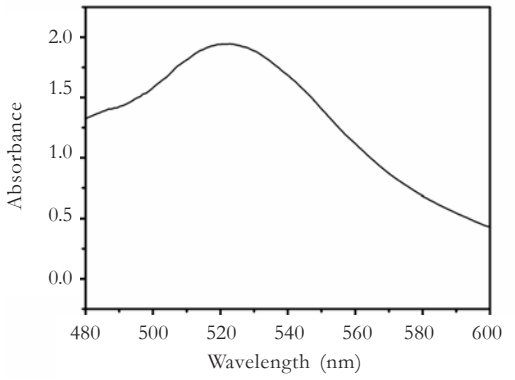

Figure 1. Absorbance spectrum of the synthesized gold solution.

The optical fibers were uncladded at the center for 1, 1.5, and 2 cm. The unclad region was incubated in APTES solution to modify the surface of the glass core fiber with the terminal amine functional group. The hydrolyzed ethoxy group of APTES reacted with the surface of glass fiber and extended the amine end chain, making it available for further reaction. The amine-terminal surface was then incubated in the gold solution, and the gold nanoparticles were reacted with the amine functional group, causing the gold to adhere on the surface of the glass fiber. This adhesion was due to the strong affinity between the positive charge of amine functional group and the negative charge of gold nanoparticles, which generated a sufficient electrostatic interaction. The schematic of the gold nanoparticles that coated the glass core of the optical fiber is

shown in Figure 2a. The gold-coated optical fiber can be seen by the naked eye from the color change at the unclad region of the optical fiber, with the result shown in Figure 2b.

The topographical and elemental information of the gold-coated optical fiber and bare glass core of the optical fiber were observed by field emission scanning electron microscopy (FE-SEM) and energy dispersive x-ray spectroscopy (EDS), respectively, as shown in Figure 3. The smooth surface of the optical fiber before coated with gold nanoparticles view in magnification 50000X show in Figure 3a. The optical fiber after coated with gold nanoparticles displays a spherical shape of the gold nanoparticles covering on the surface at the unclad region (Figure 3b), with an average gold particle size of ~20-50 nm and some small agglomerates being observed. From EDS results, the Au peak is presented on about 13.32%Wt of the gold-coated optical fiber (Figure 3d) when compared with the bare glass core optical fiber, where no the Au peak is observed (figure 3c). These results confirmed that the functionalization of gold onto the glass core surface performed well.

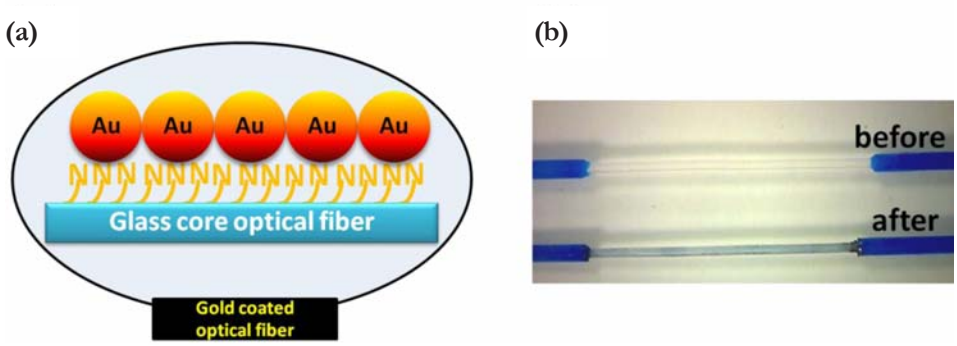

Figure 2. (a) Schematic of the gold-coated optical fiber. (b) Image of the optical fiber before (upper) and after (lower) it was coated with gold nanoparticles.

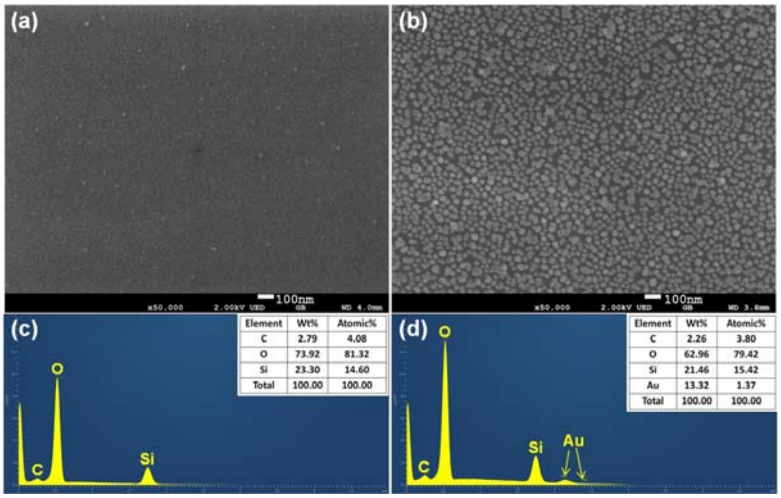

Figure 3. Topographical FE-SEM image and EDS results showing the elemental information of the optical fiber (a), (c) before and (b), (d) after it was coated with gold nanoparticles.

3.2 Refractive Index Measurements of the Gold-coated Optical Fiber

In this experiment, gold-coated optical fibers with three unclad lengths (1, 1.5, and 2 cm) were tested in glycerol solutions with different refractive index values, with the gold non-coated optical fiber used as the control condition. The signal of the fiber optic evanescent field absorbance measurements was normalized using the intensity of light measured in the glycerol solution (I_s) divided by the intensity of light measured in the water or blank solution (I_r).

The light attenuation at an absorbing wavelength is defined by the following equations [9-11],

$$-\log \frac{I_s}{I_r} = \eta_p \alpha L + \log \frac{NA_r^2}{NA_s^2} \quad (1)$$

and

$$\eta_p = \frac{k\lambda}{2\pi r NA_s}, \quad (2)$$

where η_p is the fraction of the total light intensity in the evanescent field, k is a constant, λ is the wavelength of light, r is the radius of the fiber, α is the absorptivity of the gold-coated fiber, L is the fiber length, NA_r is the numerical aperture of cladding bare fiber, and NA_s is the numerical aperture of unclad optical fiber coated gold nanoparticles. $NA_r = \sqrt{n_1^2 - n_2^2}$ and $NA_s = \sqrt{n_1^2 - n_m^2}$, where n_1 is the refractive index of the core fiber, n_2 is the refractive index of cladding of the bare fiber in blank solution, and n_m is the refractive index of the gold-coated fiber immersed in the sample solution.

The refractive index dependence involves the absorptivity, with the second term explaining the light loss, as shown in equation 1. The first term shows that the light intensity decreases by the absorption of light in the cladding through the evanescent field. This absorbance depends on the absorptivity, which increases for a higher refractive index, a greater concentration of gold nanoparticles coated on the fiber, a longer fiber length, and a smaller fiber radius [10,12]. Figure 4 shows the measurement setup of the sensor, which consists of a light source and a spectrometer that are connected by an optical fiber, with the data signal transferred to the computer.

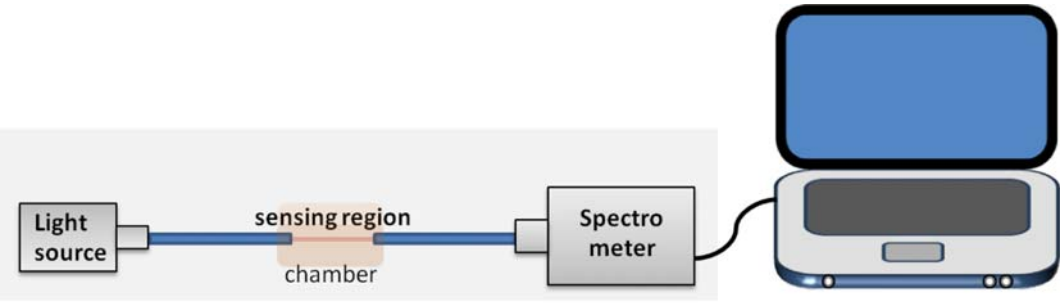

Figure 4. The setup of the gold nanoparticle-based fiber optic sensor.

The gold-coated optical fiber was used to measure the glycerol solution from 0.5-10% (v/v), where the glycerol concentration or refractive index of the solution increased the light intensity, which led to a decrease in the transmitted optical signal, with the transmitted light spectrum shown in Figure 5. The changes in the transmitted light spectrum of the gold non-coated optical fiber did not depend on an increase (or decrease) in the surrounding refractive index due to the fact that the LSPR phenomenon

was not occurring in the optical fiber (Figure 5a). However, the signal from the transmitted light spectrum of the gold-coated optical fiber decreased when the refractive index increased (Figure 5b). These results were caused by the absorption of light into the fiber through the evanescent field that was enhanced by the gold coating on the surface and increased by the higher surrounding refractive index.

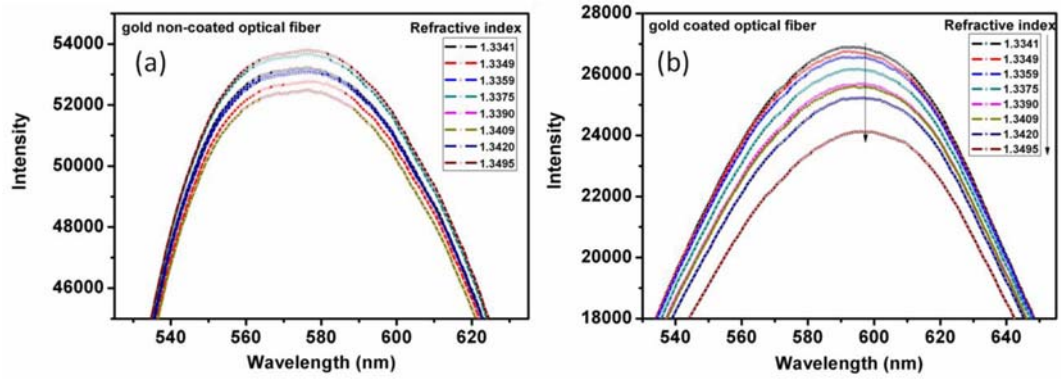

Figure 5. The light intensity of (a) the gold non-coated optical fiber and (b) the gold-coated optical fiber for different refractive index values in glycerol solution.

Here the effectiveness of the gold-coated optical fiber at different unclad lengths, 1, 1.5, and 2 cm, was determined via a signal intensity experiment in glycerol solution. The gold non-coated optical fibers were used as the control condition. The signal intensity was normalized with the blank solution to obtain the base line intensity, with the baseline then recorded when the optical fiber was immersed in the PBS solution. The normalized intensity of each sensor was plotted versus refractive index, which is shown in Figure 6. The signal of each gold-coated optical fiber decreased as the refractive index increased, which was caused by the increased absorption of evanescent field due to the gold-coated surface at the higher refractive indices. But the transmitted light spectrum of the gold non-coated optical fiber did not depend on an increase (or decrease) in the surrounding refractive index. Each unclad gold-coated optical fiber possessed a different sensitivity, which is shown in Figure 6, where the sensitivity of the unclad fiber is defined by the slope of the normalized intensities plotted as a function of refractive index. The 2 cm unclad optical fiber showed the highest sensitivity, 14.30 a.u./RIU, with the 1 and 1.5 cm unclad fibers possessing lower sensitivities, at 5.20 and 7.75 a.u./RIU, respectively. However, no trend in the signal changes was observed for the gold non-coated optical fibers as the refractive index of the solutions increased. The sensor resolution can be calculated as three times the standard deviation (3 × S.D.) of the noise signal divided by the sensitivity of each gold-coated optical fiber, which is 4.80×10^{-4}, 2.33×10^{-4}, and 9.80×10^{-5} RIU for the 1, 1.5, and 2 cm unclad optical fibers, respectively.

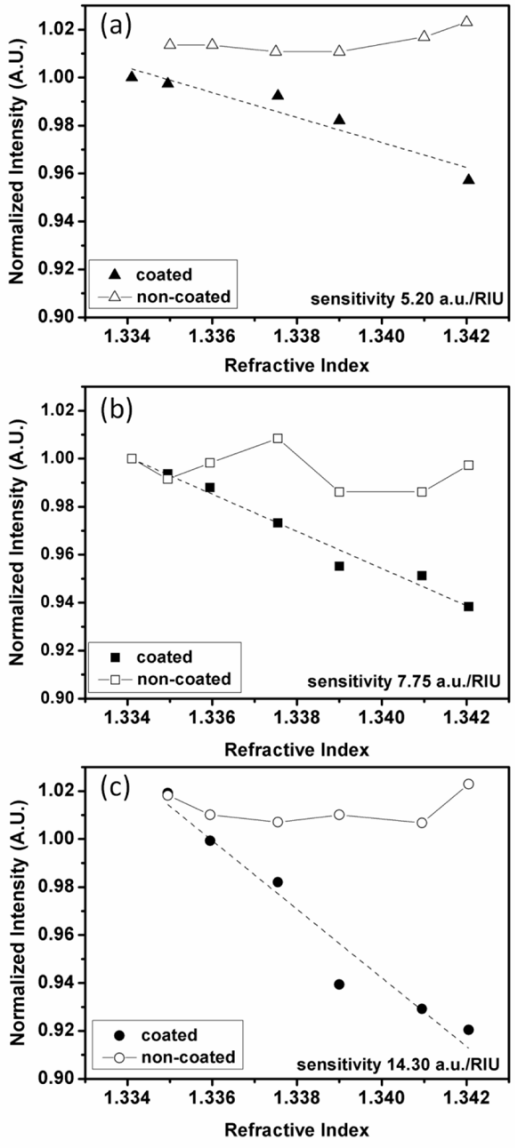

Figure 6. The normalized intensities of the non-coated and gold-coated optical fiber for a range of refractive index values and the following unclad lengths: a) 1 cm, b) 1.5 cm, and c) 2 cm.

It was observed that the sensitivity of the optical fiber increased when the unclad region of the optical fiber was increased. This is due to more gold adhering onto the fiber with the longer unclad length, which then has a larger evanescent field effect on the signal. However, although the 2 cm unclad length provided the best sensitivity, the analyzed data only had an fit of 0.94, which is lower than the fit of 0.97 for the 1.5 cm unclad length. This may be due to the non-uniformity of the gold nanoparticles coated on the glass core fiber. We therefore investigated the ability of these two unclad gold-coated optical fibers to measure the biomolecule.

3.3 Biomolecular Detection

If the LSPR sensor can detect the refractive index change of the environmental solution, then the analyte molecule can be measured without labeling. The gold-coated optical fibers with unclad lengths of 1.5 and 2 cm were used to test the feasibility of sensing this biomolecule interaction. Antibody-A was immobilized on the sensor surface for the detection of the analyte, antigen-A, on the red blood cell samples.

The results showed that the two unclad lengths, 1.5 and 2 cm, could detect the antigen on red blood cell A (A-RBCs), which had a higher signal change than that of red blood cells O (O-RBCs) and B (B-RBCs). This is due to the specific interaction of the immobilized antibody-A and the antigen-A analyte in solution, which causes an increase in the refractive index due to the presence of analyte molecules on the sensor surface; the results are shown in Figure 7. A comparison of the 1.5 and 2 cm unclad lengths indicates that the 2 cm unclad optical fiber possessed a higher specific signal for A-RBCs (7.7 Δ%T) than that of the 1.5 cm unclad optical fiber (3.4 Δ%T), but the non-specific signal with O-RBCs also increased. These higher specific signals are due to the longer gold-coated area, which had a higher sensitivity for detection. The specific signal of antibody-A and antigen-A on the A-RBCs was then compared to the non-specific signal

of antibody-A with O-RBCs and B-RBCs for the 2 cm unclad length optical fiber. The non-specific signal was defined as the noisy signal around ±1.1%T, which indicated a signal to noise (S/N) ratio of 7. However, the non-specific signal noise of the 1.5 cm unclad length optical fiber was around ±0.3%T, with a S/N ratio of 11.3, which is better than that of the 2 cm unclad length.

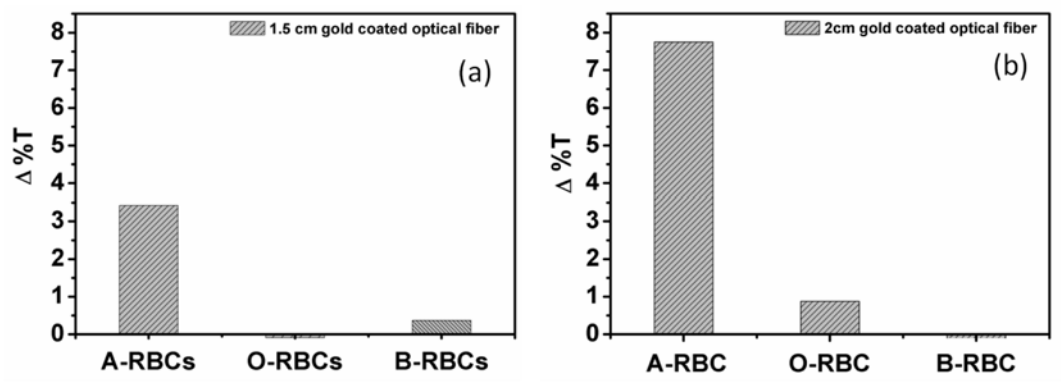

Figure 7. The signal change from the interaction of immobilized antibody-A and antigen on red blood cell groups A, B, and O for: (a) a 1.5 cm unclad optical fiber and (b) a 2 cm unclad optical fiber.

Both the 1.5 and 2 cm unclad optical fibers, modified with gold nanoparticles, can be used for detecting the interaction of immobilized antibody-A and antigen-A in solution. However, some non-specific binding signals may occur due to the physical adsorption of the red blood cell on the surface that can be washed from the surface with a mild base solution. This signal can therefore be set as the noise signal that is detected by this sensor, with a comparison of the S/N ratio between the 1.5 and 2 cm unclad fibers conducted to determine the best unclad length of the gold-coated optical fiber.

4. CONCLUSIONS

Here we demonstrated that an optical fiber can be modified with gold nanoparticles to fabricate a label-free bio-sensing device that has the advantages of a simple implementation and setup. Gold-coated optical fibers with different unclad lengths were analyzed to optimize the sensitivity of the sensor. Unclad lengths of 1-2 cm were investigated, with the longer unclad length possessing ahigher sensitivity due to a larger area of gold coated onto the surface, which provided a higher evanescent field effect. However, the S/N ratio is key parameter in determining the effectiveness of a biomolecular detection platform. The optical fiber with an unclad length of 1.5 cm provided a better S/N ratio for the detection of antigen-A on the red blood cell surface than the 2 cm unclad fiber. This gold-coated optical fiber, with the 1.5 cm unclad length, has the potential to serve as a portable device for the detection of biomolecules where a compact size is necessary.

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