Biosensors and Bioelectronics 24 (2009) 1610–1614

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Localized surface plasmon coupled fluorescence fiber-optic biosensor for alpha-fetoprotein detection in human serum

Ying-Feng Chang[a], Ran-Chou Chen[b,c], Yi-Jang Lee[b], Shu-Chen Chao[d], Li-Chen Su[e], Ying-Chang Li[e], Chien Chou[a,b,e,f,*]

[a] Institute of Biophotonics, National Yang Ming University, Taipei 112, Taiwan
[b] Institute of Biomedical Imaging and Radiological Sciences, National Yang Ming University, Taipei 112, Taiwan
[c] Department of Radiology, Taipei City Hospital Renai Branch, Taipei 106, Taiwan
[d] Division of Serology & Immunology, Taipei City Hospital, Taipei 106, Taiwan
[e] Department of Optics and Photonics, National Central University, Jung-li 320, Taiwan
[f] Department for Education and Research, Taipei City Hospital, Taipei 145, Taiwan

ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form 25 July 2008
Accepted 13 August 2008
Available online 22 August 2008

Keywords:
Fiber-optic biosensor
Gold nanoparticle
Localized surface plasmon
Alpha-fetoprotein (AFP)

ABSTRACT

In this study, we demonstrated that the fiber-optic biosensor based on localized surface plasmon coupled fluorescence (LSPCF) is capable of detecting alpha-fetoprotein (AFP) in human serum. The sensitivity of LSPCF fiber-optic biosensor is not only enhanced but also the specific selectivity is improved since the fluorophores are excited by the localized surface plasmon with high efficiency. Experimentally, this fiber-optic biosensor is able to detect AFP concentration in phosphate buffered saline (PBS) solution from 0.1 ng/mL to 100 ng/mL whereas the linear relationship between the AFP concentrations and the fluorescence signals is shown. Furthermore, a linear response between the fluorescence signals and the concentrations of AFP in human serum from 2.33 ng/mL to 143.74 ng/mL is also obtained. As a result, the detection limit of the LSPCF fiber-optic biosensor on AFP detection is comparable with the conventional enzyme-linked immunosorbent assay (ELISA). Additionally, the LSPCF fiber-optic biosensor benefits on inexpensive, disposable and simpler optical geometry that can become a high efficient immunoassay comparable with the conventional ELISA and radioimmunoassay (RIA) clinically.

1. Introduction

To date, the evanescent wave excited fluorescence (EWF) fiber-optic biosensor has been widely utilized in immunoassay. It exhibits near field sensing and real-time measurement abilities, which are capable of detecting biomolecules within the interactive region produced by an evanescent wave (Taitt et al., 2005). In addition, this biosensor shows the advantages of simple optical geometry, disposable and inexpensive (Wolfbeis, 2006). However, the EWF fiber-optic biosensor suffers by lower detection sensitivity that is compared to conventional methods such as the enzyme-linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA) (Ao et al., 2006). Recently, the gold nanoparticles (GNPs) are introduced in the biosensing discipline, which becomes one of the most efficient ways to enhance the limit of detection in the biosensors (Chau et al., 2006; Hsieh et al., 2007; Matsui et al., 2005; Mao et al., 2006). For instance, Mirkin et al. proposed that the method based on GNPs could successfully detect amyloid-β-derived diffusible ligands (ADDL) in cerebrospinal fluid (CSF) at low concentration (<1 pM) able to diagnose early stage of Alzheimer's disease in vitro (Georganopoulou et al., 2005). Meanwhile, Martins and his colleagues have demonstrated by introducing GNPs in the biosensors which leads to the ability for detecting tiny amount of DNA (<1 pM) (Martins et al., 2007).

It is known that noble-metal nanoparticles (NMNPs) possess special optical properties. A collective oscillation of conduction electrons – called localized surface plasmons (LSPs) – on NMNP surface is excited when NMNP is illuminated by a laser beam with proper wavelength which is significantly larger than the radius of NMNP (Mock et al., 2003; Rechberger et al., 2003; Sonnichsen et al., 2002). Such a phenomenon results in wavelength selective absorption with extremely large molar extinction coefficients and a significant enhancement of a localized electromagnetic field near NMNP surface (Haes et al., 2004a,b; McFarland and Van Duyne, 2003). These result in surface enhanced Raman spectroscopy (SERS) and metal enhanced fluorescence (MEF) (McFarland and

* Corresponding author at: Institute of Biophotonics, National Yang Ming University, Taipei, 112 and Department for Education and Research, Taipei City Hospital, Taipei 145, Taiwan. Tel.: +886 2 28267061; fax: +886 2 28251310.
E-mail address: cchou@ym.edu.tw (C. Chou).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.08.019

Van Duyne, 2003; Zhang et al., 2005, 2007b). This localized electromagnetic field decays exponentially from the NMNP surface where the 1/*e* decay length is about 50–60 nm by calculation (Cheng and Chau, 2003; Malinsky et al., 2001). Generally speaking, LSP is strongly dependent on the dielectric constant, size and shape of NMNP and the surrounding (Kelly et al., 2003; Kottmann et al., 2001; Kyung et al., 2006). Based on localized surface plasmon coupled fluorescence (LSPCF), our previously study proposed a novel LSPCF fiber-optic biosensor on protein–protein interactions (Hsieh et al., 2007). Experimentally, we verified that the highest sensitivity of this LSPCF fiber-optic biosensor was 1 pg/mL when detecting mouse immunoglobulin G (IgG) interacting with anti-mouse IgG. To our knowledge, this is the highest sensitivity ever measured by fiber-optic biosensor on protein–protein interaction. Then LSPCF fiber-optic biosensor based on sandwich immunoassay configuration exhibits not only on high sensitivity but also on high specificity. Besides, the features of conventional fiber-optic biosensor are included in this fiber-optic biosensor too.

In this study, we applied the developed LSPCF fiber-optic biosensor on clinical diagnosis alpha-fetoprotein (AFP) detection in human serum. Generally, AFP is a 70-kDa oncofetal glycoprotein which is a tumor marker for hepatocellular carcinoma and germ cell tumors (Fu et al., 2006; Tsai and Lin, 2005; Xu et al., 2006). Normally, AFP is produced in the fetal liver while the level is gradually reduced after birth. The blood AFP is barely detectable in the healthy adults. Statistically, the AFP concentration in the serum is less than 25 ng/mL in a healthy individual (Zhu et al., 2006). The AFP concentration in blood, however, can increase in response to the induction of hepatomas or teratomas (Chou et al., 2002). In previous research, different methods were proposed in order to detect AFP concentration in human serum. Such as the piezoelectric immunosensor (Tsai and Lin, 2005; Zhang et al., 2007a; Zhu et al., 2006), fluoroimmunoassay (Ao et al., 2006; Ye et al., 2005), multiplexed protein microarrays (Master et al., 2006), messenger RNA (Weiss et al., 2006), biobarcoded nanoparticle probes (Stoeva et al., 2006), electrochemical and chemiluminescent immunosensors (Fu et al., 2006; Lin and Ju, 2005). Although these methods exhibit their sensitivity on detecting AFP level in human serum, they require complicated operating procedures and special equipment. So far, ELISA and RIA are two popular methods employed to detect AFP concentration in human serum clinically (Ao et al., 2006; Zhu et al., 2006). RIA shows ability of high detection sensitivity (<1 ng/mL or 10 pM), however, the usage of radioisotope poses a serious safety problem (Ao et al., 2006; Chou et al., 2002). On the other hand, ELISA presents lower sensitivity (~9.9 ng/mL or 140 pM) than RIA and also requires a well-trained technician in order to reduce the error during tedious washing process (Ao et al., 2006). In this experiment, we demonstrate that the LSPCF fiber-optic biosensor has the ability to detect AFP concentration in a range from 0.1 ng/mL to 100 ng/mL in phosphate buffered saline (PBS, PH 7.4) buffer. In the meantime, the LSPCF fiber-optic biosensor can diagnose AFP level in human serum too.

2. Materials and methods

2.1. Materials

Capture antibody, bovine serum albumin (BSA), GNP conjugate protein A (Au-PA, GNP diameter = 20 nm), phosphate buffer saline (PBS) tablet, ethyl acetate and 2-propanol were purchased from Sigma Inc. (St. Louis, MO, USA). The PBS solution (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) was prepared by one tablet dissolved in 200 mL of tri-distilled water in this study. Target antigen (AFP) and secondary antibody were purchased from Calbiochem Co. (Darmstadt, Germany) and GeneTex Co. (San Antonio, TX, USA), respectively. Fluorescent labeling kit (DyLight^{TM} 649) was purchased from Pierce Co. (Rockford, IL, USA). Plastic optical fiber (polymethyl methacrylate, PMMA) of 1000 μm in diameter was purchased from Mitsubishi Rayon Co., LTD. (Tokyo, Japan). Human serums were obtained form Division of Serology & Immunology, Taipei City Hospital (Taipei, Taiwan).

2.2. Surface modification of the PMMA fiber

In this experiment, the polymethyl methacrylate (PMMA) fiber was declad by immersing the fiber in ethyl acetate. Subsequently, the optical fiber's declad portion was washed with 2-propanol to clean the surface. The declad area was 1.4 cm^2. A chemical adsorption method was used by covalent binding force in order to immobilize the capture antibody on the surface of declad portion. The protocol of chemical adsorption was described previously (Chou et al., 2007; Henry et al., 2000; Hsieh et al., 2007).

2.3. Biorecognition molecules and sandwich immunoassay

The fiber-optic biosensor is constructed based on sandwiched biomolecular complex (capture antibody/target antigen/secondary-antibody (fluorescence probe)) as shown in Fig. 1. The capture antibody belongs to the mouse monoclonal anti-AFP (isotype: IgG2a, Clone No.: C3, Sigma Co., St. Louis, MO, USA). It is immobilized on the modified declad portion through preceding chemical adsorption. In order to avoid the non-specific binding of fluorescence probe on the modified declad portion during measurement, fibers are immersed in BSA solution (10 mg/mL), in which BSA quenches the modified declad portion activation prior to incubating with target antigens.

AFP (Calbiochem Co., Darmstadt, Germany) (target antigen) was prepared at the concentrations 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL prepared in 1 mL PBS solution. The target antigen solution with 1 mL was injected into reaction chamber interacting with the immobilized capture antibody at 4 °C for 1 h to form the complex (capture antibody (anti-AFP)/target antigen (AFP)) on the modified declad portion. To measure the background noise of the fiber-optic biosensor, a 10 ng/mL non-specific BSA solution is added to the reaction chamber for data recording. In addition, we utilized 0.6 mL of fresh serums instead of target antigen solutions. The experimental procedure as described above was followed.

2.4. Preparation of the fluorescence probe

The fluorescence probe is composed of fluorophores, which are labeled with secondary-antibodies connecting to GNP-conjugated protein A (Au-PA, diameter = 20 nm, Sigma Co., St. Louis, MO, USA) as shown in Fig. 1. The secondary antibody fluorescently labeled utilizing a commercial labeling kit (DyLight^{TM} 649) is mouse monoclonal anti-AFP (isotype: IgG1, Clone No.: F1, GeneTex Co., San Antonio, TX, USA). To produce the fluorescence probe, the fluorophore labeled anti-AFP is mixed with Au-PA, which has a concentration of 1.25×10^9 particles/mL. In this experiment, the concentration of fluorophore labeled anti-AFP was 1 μg/mL, and incubation time was 2 h under the condition at 4 °C in a dark environment.

2.5. Basic principle and experimental set-up

This fiber-optic biosensor combines LSPCF technique with sandwich immunoassay (Hsieh et al., 2007). The sandwich structure is built on the modified declad portion of the PMMA fiber. LSPs which are excited by evanescent wave produce a significant enhancement

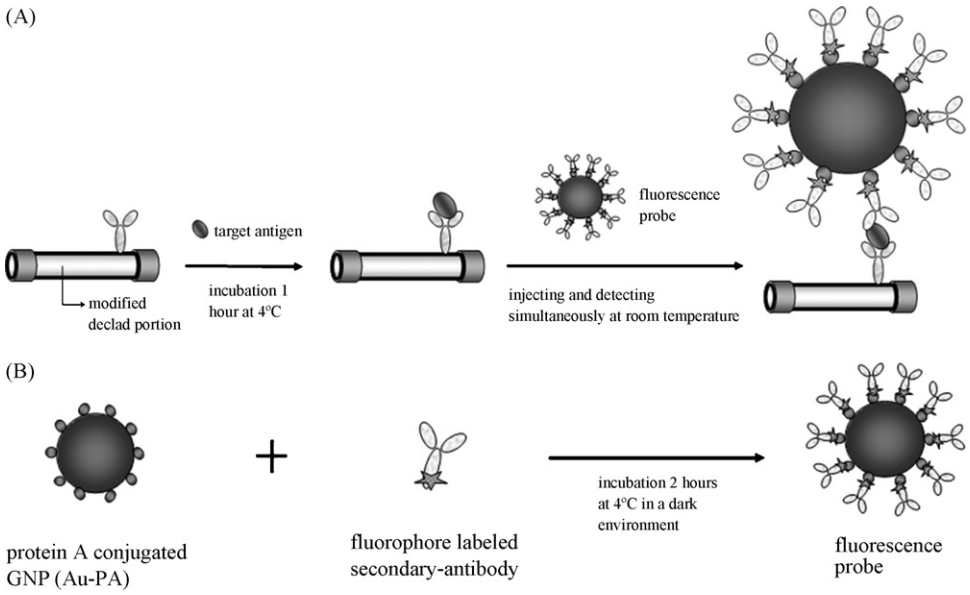

Fig. 1. (A) Scheme of declad portion and sandwich complex. (B) Scheme of fluorescence probe.

of the localized electromagnetic field near GNP surface. Thereafter the fluorophores labeled secondary-antibodies are excited by enhanced localized field of LSPs.

The experimental set-up is performed as shown in Fig. 2 in which a 658 nm diode laser was used as the excitation light source. To achieve high coupling efficiency of laser beam into multimode optical fiber (1 mm in diameter, NA = 0.467), a 20× microscopic objective of NA = 0.45 is used. There are multiple modes of laser beam to propagate in the fiber that is able to produce a uniform intensity distribution of the evanescent wave on the modified declad portion surface. In measurement, the fluorophore was excited by using laser beam (658 nm) from laser diode. The central wavelength of the emission spectrum is 674 nm and the FWHM of this spectrum is about 70 nm. A narrow band interference filter which the center wavelength locates at 680.4 nm and the bandwidth is 11.1 nm is introduced in this instrument for fluorescent detection via a photomultiplier tube (PMT). In the experiment, a lock-in amplifier was utilized to increase the signal-to-noise ratio (SNR).

3. Results

The temporal fluorescence intensity of biomolecular interaction between the fluorescence probe and AFP at different concentrations in PBS solution is illustrated in Fig. 3. The concentrations of AFP were at 100 ng/mL, 10 ng/mL, 1 ng/mL, 0.1 ng/mL. A fixed concentration of fluorescence probe was arranged by fluorophore labeled anti-AFP (1 μg/mL) conjugated with Au-PA (1.25×10^9 numbers/mL). In order to measure the background noise of the fiber-optic biosensor, 1 mL non-specific solution (10 ng/mL BSA in PBS solution) was added to the reaction chamber for recording. The unit of fluorescence intensity is an arbitrary unit (a.u.) defined as the fluorescence intensity normalized by the intensity of laser beam.

The relationship between the fluorescence signal and the AFP concentration (in logistic scale) over the range from 0 ng/mL to 100 ng/mL is linear as shown in Fig. 4. The correlation coefficient (R^2) is 0.9868 and the error bar is equivalent to one standard derivation in this experiment. The fluorescence signal is obtained by

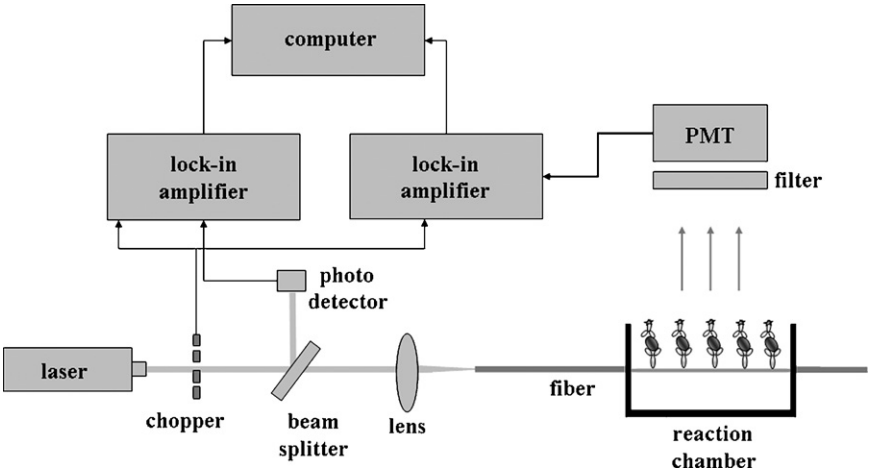

Fig. 2. Scheme of experimental set-up. PMT: photomultiplier tube.

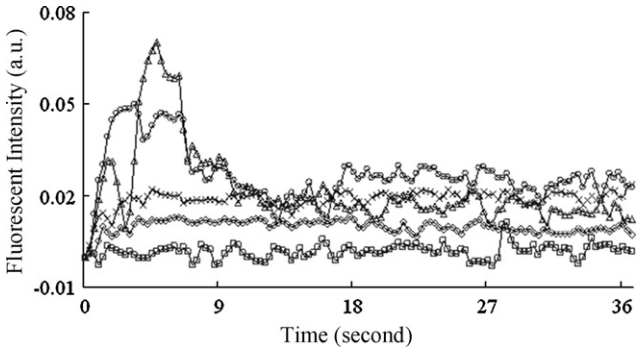

Fig. 3. The binding processes of fluorescence probe interaction with different concentrations of target antigen. [Square, 10 ng/mL non-specific BSA; diamond, 0.1 ng/mL APF; triangle, 1 ng/mL AFP; crisscross, 10 ng/mL AFP; circle, 100 ng/mL AFP]. The uncertainty of the measurement is smaller than the size of data points in this figure.

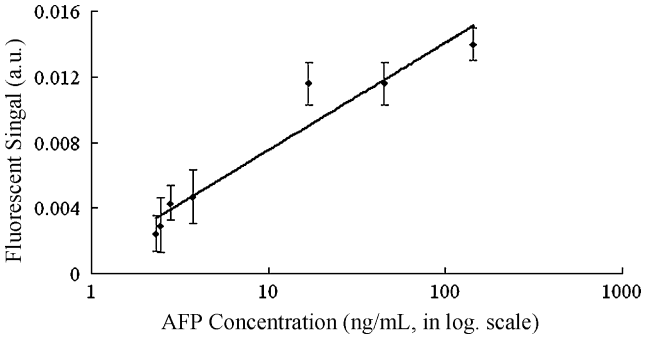

Fig. 5. The linear relationship [$y = 0.0028\ \mathrm{Ln}(x) + 0.0010$; $R^2 = 0.9331$] of different human serum versus fluorescent signal. The error bar is equivalent to one standard derivation in this experiment. The error percentages are 2.33 ng/mL: 44.84%, 2.45 ng/mL: 57.03%, 2.79 ng/mL: 24.16%, 3.73 ng/mL: 34.51%, 16.75 ng/mL: 11.06%, 44.93 ng/mL: 11.18%, 143.74 ng/mL: 6.91%.

subtracting background level from the fluorescence intensity in this experiment. A similar relationship between the fluorescence signal and a logistic scale of IgG at low concentrations has been demonstrated (Hsieh et al., 2007).

To demonstrate LSPCF fiber-optic biosensor is suitable for clinical application, human serums are used for detection of AFP level instead of AFP-PBS solutions. It has been reported that human serums with AFP concentration ranging from 2.33 ng/mL to 143.74 ng/mL are measurable by commercial ELISA system (Architect i2000, Abbott, Abbott Park, IL, USA). Our result demonstrated that a correlation between the fluorescent signals and the concentration range described previously is well performed as shown in Fig. 5. The correlation coefficient (R^2) is 0.9331 and the error bar is defined one standard deviation in measurement. The net fluorescence signal is derived by subtracting the background level from the fluorescence intensity.

4. Discussion

There are three parts of the detected signal versus time in Fig. 3 at different concentration. (a) Region I (0–5 s), region II (5–9 s) and region III (after 9th second). In region I, we injected fluorescence probes into the reaction chamber, and then numerous fluorescence probes rush into the interaction region of evanescent field. This results in the fluorescence signal growth so rapidly at beginning. However, not all fluorescence probes in the interaction region are bound with antigen which is captured by immobilized capture antibody. Then, part of those fluorescence probes which are not bound by antigen diffuse out of the region of evanescent field that causes the fluorescence signal decreases apparently as shown in region II. Finally, the fluorescence signal becomes saturated when the biomolecular interaction reaches an equilibrium state as shown in the region III. In Fig. 3, SNR of different concentrations is SNR > 5 except the non-specific BSA solution which is defined as background noise. The mean value over the last 20 data points and the standard deviation of these data points are described as the signal and noise, respectively.

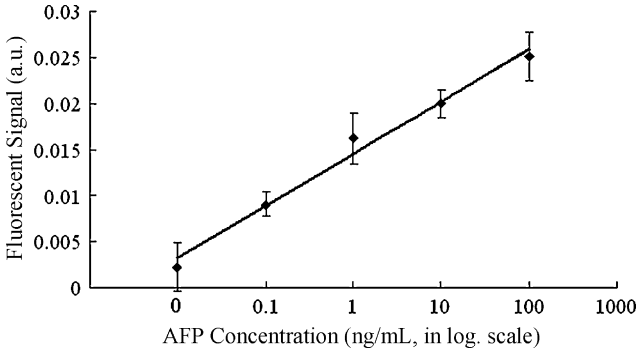

Fig. 4. The linear relationship [$y = 0.0025\ \mathrm{Ln}(x) + 0.0145$; $R^2 = 0.9868$] of AFP concentration (in PBS solution) versus fluorescent signal. The error bar is equivalent to one standard derivation in this experiment. The error percentages are 0 ng/mL: 114.96%, 0.1 ng/mL: 14.04%, 1 ng/mL: 16.71%, 10 ng/mL: 7.63% and 100 ng/mL: 10.50%.

The detection limit of this proposed LSPCF fiber-optic biosensor on AFP detection is 0.1 ng/mL (1.4 pM) in PBS solution. However, in human serum, it is able to detect AFP concentration at 2.33 ng/mL. This sensitivity is comparable with other methods (Ao et al., 2006; Fu et al., 2006; Tsai and Lin, 2005; Ye et al., 2005; Zhu et al., 2006) on detection AFP in human serum. Additionally, this detection limit of LSPCF fiber-optic biosensor is also comparable with conventional ELISA (Ao et al., 2006). In case of a tapered optical fiber is considered in this LSPCF fiber-optic biosensor, the field of evanescent wave is enhanced (Leung et al., 2007), so does the sensitivity of LSPCF biosensor. In the mean time, the V number mismatch of the optical fiber will decrease the efficiency of fluorescence signal return back to fiber as well. Therefore, the fluorescence signal detected besides the optical fiber is setup enabling to result high collection efficiency. Then, to integrate tapered optical fiber into this LSPCF biosensor ensures high detection sensitivity. Finally, the LSPCF fiber-optic biosensor not only presents the advantages of inexpensive and disposable, but also provides a simple optical geometry. This makes LSPCF fiber-optic biosensor becomes easier handling and faster response on human serum detection compared with ELISA and RIA.

According to our previous study (Hsieh et al., 2007), the high sensitivity of LSPCF fiber-optic biosensor has been demonstrated based on two features. (1) The fluorescence is excited and enhanced by the LSPs on GNP efficiently. (2) The fluorescence signal is amplified because each of fluorescence probe contained 40 fluorophores which are excited simultaneously. However, the loss of fluorescence due to the metal-induced quenching effect reduces the performance of the LSPCF fiber-optic biosensor (Yu et al., 2004). To circumvent this problem, protein A conjugated on GNP is necessary that the quenching of fluorescence by GNP is avoided. Then, protein A not only plays as a linker to the secondary antibody but also as a spacer that is able to avoid the fluorescence quenching effectively.

As far as bio-specificity is concerned clinically, the LSPCF fiber-optic biosensor shows high specificity on detecting target antigen in human serum. First, the LSPs on the GNP surface are excited by the

evanescent wave, thereby producing a strong localized electromagnetic field near GNP surface within 50–60 nm in distance (Cheng and Chau, 2003; Malinsky et al., 2001). The electromagnetic field is more localized than a planar surface plasmon wave of which the decay length is approximately 150 nm (Yu et al., 2004). The localized specificity of antigen–antibody interaction is then improved. This indicates that the fluorescence signals from the fluorescence probes which are captured by antigen properly. In Fig. 3, the fluorescence intensity at zero concentration (non-specific BSA solution) is invariable during the measurement that verifies this predication. This experiment demonstrates that the non-specific binding can be ignored since only a few non-specific antigens exist in the region of LSP field. Second, the modified declad portion was quenched by use of BSA prior to incubating with the target antigens in order to prevent the modified declad portion from directly interacting with the target antigens or fluorescence probe during the measurement. Finally, this LSPCF fiber-optic biosensor is based on sandwich immunoassay, the target antigens caught by the capture antibodies are detected by the report antibodies (fluorescence probes). This means that the target antigens have two specific interactions with the antibodies (capture and report antibody) in this immunoassay. As a result, the specificity of the sandwich complex becomes much higher than other immunoassays such as direct labelling, single-capture antibody experiments (Kingsmore, 2006).

5. Conclusions

In this study, we have successfully demonstrated that the LSPCF fiber-optic biosensor is able to detect AFP concentration in human serum directly. LSPCF fiber-optic biosensor integrates a sandwich immunoassay with LSPs on the surface of GNP to improve specificity and sensitivity, simultaneously. In the experiment, a linear relationship between the fluorescence signal and the concentration of AFP in buffer solution is observed from the zero concentration (0 ng/mL) to 100 ng/mL (in logistic scale) experimentally. In the meantime, the concentration of AFP in human serum ranging from 2.33 ng/mL to 143.74 ng/mL (in logistic scale) is also seen. Both correlation coefficients are 0.9868 and 0.9331, respectively. Potentially, the LSPCF fiber-optic biosensor can be extended into multiplexed detection as well on clinical applications.

Acknowledgements

This research was supported by Taipei City Hospital through Research Grant # 96001-62-028, and National Science Council of Taiwan through Research Grant # NSC94-2120-M-010-002, NSC95-2120-M-010-002. The support from “Aim for the Top University Plan” is also appreciated.

References

Ao, L.M., Gao, F., Pan, B.F., He, R., Cui, D.X., 2006. Anal. Chem. 78, 1104–1106.
Chau, L.K., Lin, Y.F., Cheng, S.F., Lin, T.J., 2006. Sens. Actuator B: Chem. 113, 100–105.
Cheng, S.F., Chau, L.K., 2003. Anal. Chem. 75, 16–21.
Chou, S.F., Hsu, W.L., Hwang, J.M., Chen, C.Y., 2002. Clin. Chem. 48, 913–918.
Chou, C., Hsu, H.Y., Wu, H.T., Tseng, K.Y., et al., 2007. J. Biomed. Opt. 12, 024025.
Fu, Z.F., Hao, C., Fei, X.G., Ju, H.X., 2006. J. Immunol. Methods 312, 61–67.
Georganopoulou, D.G., Chang, L., Nam, J.M., Thaxton, C.S., Mufson, E.J., Klein, W.L., Mirkin, C.A., 2005. Proc. Natl. Acad. Sci. U.S.A. 102, 2273–2276.
Haes, A.J., Zou, S.L., Schatz, G.C., Van Duyne, R.P., 2004a. J. Phys. Chem. B 108, 6961–6968.
Haes, A.J., Zou, S.L., Schatz, G.C., Van Duyne, R.P., 2004b. J. Phys. Chem. B 108, 109–116.
Henry, A.C., Tutt, T.J., Galloway, M., Davidson, Y.Y., McWhorter, C.S., Soper, S.A., McCarley, R.L., 2000. Anal. Chem. 72, 5331–5338.
Hsieh, B.Y., Chang, Y.F., Ng, M.Y., Liu, W.C., Lin, C.H., Wu, H.T., Chou, C., 2007. Anal. Chem. 79, 3487–3493.
Kelly, K.L., Coronado, E., Zhao, L.L., Schatz, G.C., 2003. J. Phys. Chem. B 107, 668–677.
Kingsmore, S.F., 2006. Nat. Rev. Drug Discov. 5, 310–320.
Kottmann, J.P., Martin, O.J.F., Smith, D.R., Schultz, S., 2001. Phys. Rev. B 64, 235402.
Kyung, K.M., Kim, S.J., Kim, D.H., 2006. Appl. Opt. 45, 3382–3389.
Leung, A., Shankar, P.M., Mutharasan, R., 2007. Sens. Actuator B: Chem. 125, 688–703.
Lin, J.H., Ju, H.X., 2005. Biosens. Bioelectron. 20, 1461–1470.
Malinsky, M.D., Kelly, K.L., Schatz, G.C., Van Duyne, R.P., 2001. J. Am. Chem. Soc. 123, 1471–1482.
Mao, X.L., Yang, L.J., Su, X.L., Li, Y.B., 2006. Biosens. Bioelectron. 21, 1178–1185.
Martins, R., Baptista, P., Raniero, L., Doria, G., Silva, L., Franco, R., Fortunato, E., 2007. Appl. Phys. Lett. 90, 023903.
Master, S.R., Bierl, C., Kricka, L.J., 2006. Drug Discov. Today 11, 1007–1011.
Matsui, J., Akamatsu, K., Hara, N., Miyoshi, D., Nawafune, H., Tamaki, K., Sugimoto, N., 2005. Anal. Chem. 77, 4282–4285.
McFarland, A.D., Van Duyne, R.P., 2003. Nano Lett. 3, 1057–1062.
Mock, J.J., Smith, D.R., Schultz, S., 2003. Nano Lett. 3, 485–491.
Rechberger, W., Hohenau, A., Leitner, A., Krenn, J.R., Lamprecht, B., Aussenegg, F.R., 2003. Opt. Commun. 220, 137–141.
Sonnichsen, C., Franzl, T., Wilk, T., Plessen, G.von, Feldmann, J., Wilson, O., Mulvaney, P., 2002. Phys. Rev. Lett. 88, 077402.
Stoeva, S.I., Lee, J.S., Smith, J.E., Rosen, S.T., Mirkin, C.A., 2006. J. Am. Chem. Soc. 128, 8378–8379.
Taitt, C.R., Anderson, G.P., Ligler, F.S., 2005. Biosens. Bioelectron. 20, 2470–2487.
Tsai, W.C., Lin, I.C., 2005. Sens. Actuator B: Chem. 106, 455–460.
Weiss, H.S., Stemmer, S.M., Liberzon, E., Avigad, S., et al., 2006. Cancer Detect. Prev. 30, 204–209.
Wolfbeis, O.S., 2006. Anal. Chem. 78, 3859–3874.
Xu, Y.Y., Bian, C., Chen, S.F., Xia, S.H., 2006. Anal. Chim. Acta 561, 48–54.
Ye, Z., Tan, M., Wang, G., Yuan, J., 2005. Talanta 65, 206–210.
Yu, F., Persson, B., Löfås, S., Knoll, W., 2004. Anal. Chem. 76, 6765–6770.
Zhang, Y., Gu, C., Schwartzberg, A.M., Zhang, J.Z., 2005. Appl. Phys. Lett. 87, 123105.
Zhang, B., Zhang, X., Yan, H.H., et al., 2007a. Biosens. Bioelectron. 23, 19–25.
Zhang, Y.X., Aslan, K., Previte, M.J.R., Geddes, C.D., 2007b. Appl. Phys. Lett. 90, 053107.
Zhu, Q., Ruo, Y., Yaqin, C., Wang, N., Zhuo, Y., Zhang, Y., Li, X.L., 2006. Electrochim. Acta 51, 3763–3768.