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A Novel Optical Biosensing System Using Mach–Zehnder-Type Optical Waveguide for Influenza Virus Detection

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Abstract In order to minimize the damage from viral epidemics, early detection of the causative agent of a viral epidemic and prevention of its immediate spread are urgent social demands. Therefore, in this study, we evaluated the utility of a Mach–Zehnder-type optical waveguide as a sensing device for influenza virus detection. However, it is impossible to detect a 100-nm-size virus using a sol-gel optical biosensor because sol-gel glass has a pore size of only a few nanometers, which makes it impossible for the virus to diffuse into the silica thin film. In order to construct the influenza-specific Mach–Zehnder optical biosensor for influenza detection, a stable antibody immobilization method with resulting high density on the sol-gel surface is strongly required. In this study, the sol-gel glass surface was modified with amino and carboxyl groups, and an anti-H1N1/HA1 antibody was covalently immobilized using a cross-linking agent. We successfully prepared a carboxyl-modified sol-gel surface, using NHS/EDC as the cross-linker, for antibody immobilization, and confirmed the detection of influenza virus using the antibody-immobilized sol-gel glass. After treatment with a 100 μg/mL influenza virus solution for 15 min, a peak wavelength shift (~24 nm) was observed in the output light spectrum.

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Introduction

Influenza is a serious respiratory disease caused by the influenza virus. Every year, 20 % of children and 5 % of adults contract the flu worldwide [1], and an influenza epidemic can lead to both social and economic losses. Therefore, in order to minimize the damage from viral epidemics, early detection of the causative pathogen of a viral epidemic and prevention of its immediate spread is important. In general, diagnostic kits for the detection of the influenza virus antigens are widely available for use in clinical diagnosis [2–4]. However, the influenza virus always diffuses into the atmosphere and infects humans, who then act as an intermediary. Therefore, instead of measuring signal "points" using conventionally extracted samples, three-dimensional "multipoint" measurement is considered an essential solution for monitoring and controlling the spread of the virus. In other words, by deploying the sensor system throughout public spaces such as airports, hospitals, and schools, we can monitor the distribution and rate of spread of the virus. In this study, we evaluated the utility of a Mach–Zehnder (MZ)-type optical waveguide for the detection of the influenza virus. Sol-gel processed materials have glass-like characteristics such as transparency, homogeneity, and low optical loss; moreover, their refractive index can be easily controlled, which allows the transmission of optical signals based on the same principle as optical fibers. Sol-gel silica thin film, which has a porous structure, is capable of reacting with molecular recognition elements within the glass, and high-sensitivity sol-gel optical biosensors have been reported [5–7]. However, it is impossible to detect a 100-nm virus using a sol-gel optical biosensor because sol-gel glass has a pore size of only a few nanometers, which makes it impossible for the virus to diffuse into the silica thin film. In order to extend optical biosensing technology, antibodies need to be immobilized on the sol-gel surface, and it is necessary to detect the changes in light intensity that occur when the refractive index changes in response to the molecular density change caused by antigen capture. Therefore, a stable antibody immobilization method with resulting high density on the sol-gel surface is strongly required. The MZ sol-gel silica waveguide was fabricated using a previously demonstrated process for biophotonic sensors and hybrid electro-optic polymer/sol-gel silica waveguide modulators [8]. The MZ interferometer waveguides have been employed for biosensors and index sensors [9–11]. Even though Si and polymer materials were used to fabricate the sensors based on an interferometric MZ waveguide, the sol-gel silica waveguide and other glass waveguides have advantages because of their lower coupling loss (e.g., <0.1 dB) between a standard optical fiber and the waveguide, which is important for multiple and in-line connection of the biosensor waveguides. When the biosensor waveguides were connected using standard optical fibers, the sensing range was dramatically extended. Moreover, living materials for the biosensor can be doped directly into the sol-gel waveguide core, and the sensing materials can be accessed by the living materials through the mesoporous structure of the sol-gel core. The sol-gel silica waveguide improved sensitivity of the sensors because the optical intensity inside the core is higher than that in the waveguide cladding by two to three orders of magnitude. Here, we show real-time biophotonic sensing detection for the virus (H1N1/HA1) based on the MZ sol-gel silica waveguide sensors. The reaction between antigen-antibody was confirmed form the sol-gel silica surface itself and sifts of an optical output spectrum from the MZ waveguide sensor.

Materials and Methods

Materials

3-Aminopropyl triethoxy silane (APTES), disodium hydrogen phosphate, ethanol, 1-hydroxycyclohexyl phenyl ketone (Irgacure 184), polyoxyethylene (20) sorbitan monolaurate (Tween 20), sodium chloride, sulfuric acid, and toluene were obtained from Wako Pure Chemical (Osaka, Japan). Blocking One, enzyme-linked immunosorbent assay (ELISA) POD substrate 3,3′,5,5′-tetramethylbenzidine (TMB) kit, glutaraldehyde (GA; 25 % in water), hydrochloric acid, isopropyl alcohol, potassium chloride, and potassium dihydrogenphosphate were obtained from Nacalai Tesque (Kyoto, Japan). Methacrylic acid, N-hydroxy succinimide (NHS), sodium hydroxide, 3-(trimethoxysilyl) propyl methacrylate (98 %), and zirconium (IV) propoxide (ZrPO; 70 wt% solution in 1-propanol) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP)-conjugated anti-influenza A was obtained from Virostat (Portland, ME, USA). 2-Carbomethoxy ethyl trichlorosilane (CMETS) was obtained from Gelest (Morrisville, PA, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was obtained from Dojindo (Kumamoto, Japan). The capture antibody, monoclonal anti-human H1N1/HA1 antibody (clone AT1G7), was obtained from ATGen (Seongnam, Gyeonggi-do, South Korea). Native influenza A H1N1 antigen was obtained from AbD Serotec (Kidlington, UK). Anhydrous sodium dihydrogen phosphate was obtained from Kishida Chemical (Osaka, Japan).

Antigen-Antibody Reaction on Sol-Gel Silica Surface

Sol-gel solutions of 3-methacryloyloxy propyltrimethoxylane (MAPTMS)/ZrPO at molar ratios of 85:15 and 88:12 were prepared for the sol-gel core and cladding, respectively. Subsequently, the mixture was stirred for 80 min and stored at 4 °C for 1 day. A 25×25-mm glass slide (Matsunami, Osaka, Japan) was cut using a glass cutter (Konyo, Niigata, Japan). The cut slide glass was washed in ethanol with sonication for 10 min. Of the sol-gel solution, 1 mL was filtered using a Millex®-LG 0.2-μm filter unit (EMD Millipore, Billerica, MA, USA), dropped on the cut glass slide, and spin coated at 4500 rpm for 45 s using an Opticoat Spin Coater (Mikasa, Tokyo, Japan). The sol-gel-coated glass was stiffened by irradiation with UV light (365 nm, 350 $\mu W/cm^2$) for 20 min.

Hydroxyl group-modified sol-gel-coated glass was prepared by O_2 plasma treatment (100 W, 1 min, 30 Pa) using a FEMTO-Q (Diener Electronic, Ebhausen, Germany). To modify with amino groups, the hydroxyl-modified sol-gel glass was stirred for 2 h at room temperature in 1 % APTES solution, washed with toluene, and dried with N_2. The glass was dried for 2 h at 110 °C, washed with ethanol and ultrapure water, and dried with N_2. To modify with carbonyl groups, aminosilane-modified sol-gel-coated glass was soaked in a 2 mL of a 10 % GA solution in a Corning suspension culture dish (CORNING®, Corning, NY, USA), stirred for 30 min at room temperature, and washed with 0.1 M phosphate buffer (pH 7.0). To immobilize the capture antibody, 5 μL of 10 μg/mL influenza A (H1N1) HA antibody solution was spread over the glass surface, incubated for 1 day at 4 °C, and washed with 10 mM phosphate buffer (pH 8.6). To block nonspecific adsorption, the capture antibody-immobilized sol-gel-coated glass was soaked in 5× Blocking One solution for 1 h and subsequently washed with phosphate-buffered saline with Tween 20 (PBS-T). Inactivated influenza A (H1N1) virus

(5 μL of 10 μg/mL) was added onto the sol-gel-coated glass and incubated for 1.5 h at room temperature. HRP-conjugated influenza A (H1N1) antibody (5 μL of 1 μg/mL) was added onto the antigen- and antibody-immobilized sol-gel-coated glass and incubated for 20 min at room temperature, and the HRP marker detective antibody was then immobilized. Finally, the glass was washed with PBS-T and then incubated with the TMB substrate according to the manufacturer's instructions.

Hydroxyl group-modified sol-gel-coated glass was prepared by the procedures mentioned above. Subsequently, the glass was washed with ethanol and ultrapure water and dried with N_2. For ester group modification, the glass was soaked in 0.5 mM CMETS at 4 °C for 1 h, washed with toluene, and dried with N_2. Subsequently, the glass was washed with ethanol and ultrapure water and dried with N_2. The ester group-modified sol-gel glass was soaked in 1 M HCl for 2 h at 70 °C to immobilize the carboxyl group onto the glass; subsequently, the glass was washed with ultrapure water and dried with N_2. In order to activate the carboxyl groups, the carboxyl group-modified sol-gel-coated glass was soaked in a 2 mL of 0.1 M EDC/0.05 M NHS in a petri dish and stirred at room temperature for 30 min. Subsequently, the glass was washed with 10 mM PBS (pH 7.4) and dried with N_2. Then, a 5 μL of 10 μg/mL influenza A (H1N1) HA antibody solution was spread onto the glass surface and incubated at 4 °C for 1 day. The capture antibody was immobilized onto the glass through amide bond formation between the activated carboxyl group on the glass and branched-chain amino groups in the antibody. Finally, the glass was washed with 10 mM phosphate buffer (pH 6.6) and incubated with the TMB. When HRP reacts with H_2O_2, TMB is oxidized to form a blue-colored compound. Then, oxidation is terminated by addition of an acid or other appropriate terminator; upon termination, the TMB color changes to yellow and absorbance can be detected at 450 nm. HRP-conjugated secondary antibody-immobilized sol-gel-coated glass was incubated in 300 μL of the TMB reaction mixture for 20 min at room temperature. The reaction was terminated by adding 150 μL of 1 M H_2SO_4 mixture. Immediately afterward, absorbance at 450 nm was measured.

Biophotonic Sensor to Detect H1N1/HA1 Virus Based on the Mach–Zehnder Sol-Gel Silica Waveguide

A sol-gel solution was prepared for the waveguide core, overcladding, side cladding, and undercladding layers and consisted of MAPTMS and an index modifier (zirconium(IV)-*n*-propoxide). The sol-gel silica solutions for the core and the claddings were doped with the index modifier with a molar ratio of 85(MAPTMS)/15, and 95(MAPTMS)/5 mol%, respectively. A 0.1 N HCl solution was used as a catalyst, and Irgacure 184 (Ciba Specialty Chemicals, Basel, Switzerland) was used as a photoinitiator for the core and the side cladding solutions to accelerate hydrolysis of the silica for subsequent wet etching in isopropanol. A 4-μm-thick undercladding was coated on silica (6-μm)-on-silicon substrate and baked at 150 °C for 1 h. A 4-μm-thick sol-gel silica core layer was spin coated on the undercladding. Mercury i-line (365-nm) radiation was delivered from a mask aligner to the sol-gel layer through a photomask to etch a 4-μm-wide core region. The regions exposed to radiation were cross-linked to form a silica network. The ultraviolet-irradiated areas became insoluble in isopropanol, which was used as a wet etchant. After wet etching the core, side cladding was spin coated and baked at 150 °C for 1 h. The solution of the overcladding was spin coated and wet etched only for one arm of the MZ waveguide core surface (Fig. 1) to expose to the virus using the same method as that for the

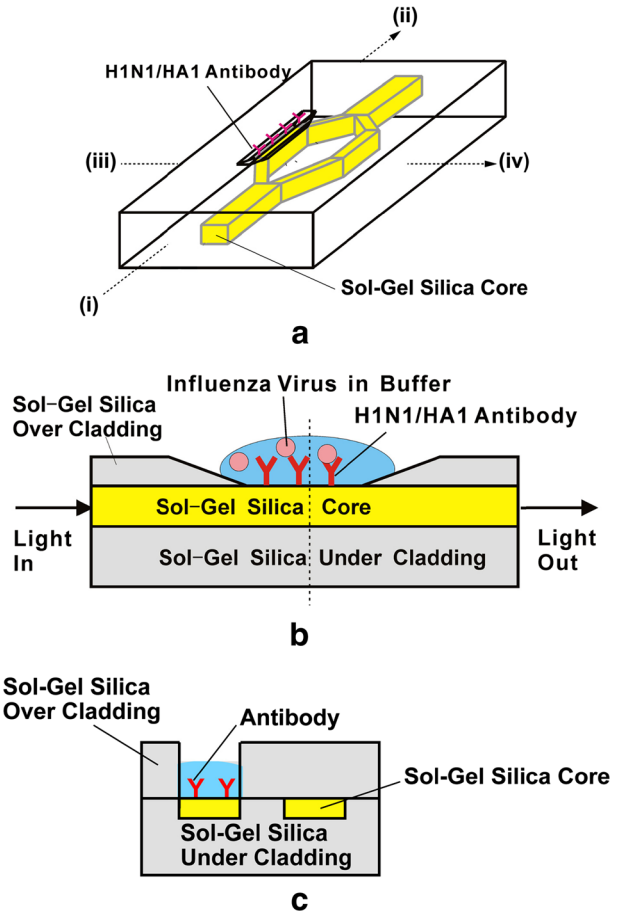

Fig. 1 Schematic of **a** the Mach–Zehnder sol-gel silica waveguide biophotonic sensor, **b** longitudinal section of the device (*i* and *ii*), and **c** cross section of the device (*iii* and *iv*)

core, which worked as a biosensing window. The overcladding had a vertical taper structure (taper angle<4°) in the window to reduce an optical loss when the virus buffer solution was dropped on the MZ window. Finally, the waveguide was hard baked at 150 °C for 1 h. After the waveguide fabrication, a white light source at the wavelengths from 1520 to 1620 nm (ASE-FL7002, FiberLabs Inc., Fujimino, Saitama, Japan) was coupled to the MZ waveguide through a standard optical fiber (SMF-28, Corning Inc., NY, USA) as shown in Fig. 2. Output power from the MZ waveguide was coupled with another single-mode fiber and monitored with an AQ6319 optical spectrum analyzer (OSA; Yokogawa Electric Corp., Tokyo, Japan). After the virus solution (10 μL of 100 μg/mL (number of virus particle 1.0×10^{10})) was dropped on the waveguide surface, the output spectrum was continuously monitored on the OSA. We used

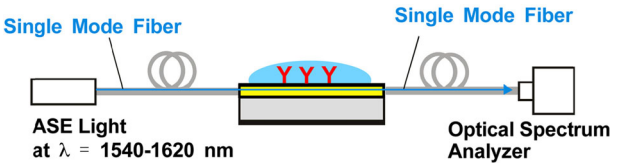

Fig. 2 Experimental setup to detect virus based on the sol-gel silica Mach–Zehnder waveguide

standard single-mode fiber with a coupling loss of <1 dB, which can be applied for distributed fiber sensor network when the fiber was expended for the length of >50 km. The propagation loss of the waveguide was 2 dB/cm at the wavelength of 1550 nm.

Results and Discussion

In order to construct the MZ optical biosensor for the detection of the influenza virus, an H1N1/HA1 antibody needs to be immobilized onto the sol-gel glass surface, necessitating the introduction of a functional group onto the surface. In this paper, the sol-gel glass surface was modified with amino and carboxyl groups, and the antibody was covalently immobilized using a cross-linking agent (Fig. 3). Figure 4a shows the results of the antibody-antigen reaction caused by amino group modification using GA as the cross-linking agent; no specific response due to the presence or absence of the antigen was observed. This result is because the aminosilane layer, which was introduced onto the sol-gel surface by APTES, formed a multilayer structure [12] and the GA binding site was oriented in different directions (Fig. 3a); consequently, the supplementary antibody was sterically hindered and was not immobilized. Figure 4b shows the results of the antibody-antigen reaction prepared by carboxyl group modification using NHS/EDC as the cross-linking agent; in this case, the antigen-antibody reaction was confirmed. This is because the carboxyl groups, which were introduced onto the surface by hydrolysis with HCl, formed a monomolecular layer [13]. As a result, compared with the amino group-modified sol-gel glass, the antigen recognition site of the supplementary antibody was maintained (Fig. 3b) and the accessibility of antigen-antibody reaction increased. Hence, it is concluded that the carboxyl-modified sol-gel glass is suitable for the antigen-antibody reaction. Then, we evaluated the sensor response associated with influenza binding using an MZ-type sol-gel optical waveguide.

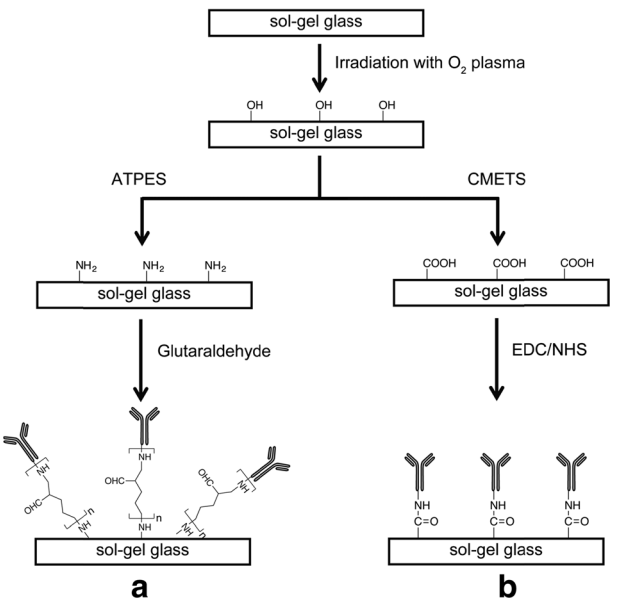

Fig. 3 Schematic illustration of immobilization of antibody onto sol-gel glass surface **a** amino group-modified substrate and **b** carboxyl group-modified substrate

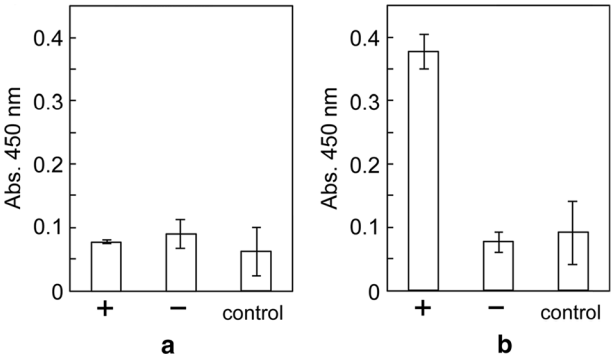

Fig. 4 Evaluation of antibody-antigen reaction; presence of antigen (*plus sign*), absence of antigen (*minus sign*), and non-treated sol-gel glass (control). **a** Amino groups introduced and **b** carboxyl groups introduced

In this study, we did not dope the antibody inside the sol-gel silica core. Instead, we put the antibody on the surface on one arm of the MZ waveguide branch and other arm was covered by the sol-gel silica cladding as shown in Fig. 1. Therefore, we could detect the change in the refractive index of one MZ arm due to the reaction between the antibody and the virus from the change of output power. The optical spectrum of the output power from the MZ waveguide is shifted due to the change in the refractive index of the MZ arm. The antibody was attached to the MZ waveguide after the waveguide was treated and cleaned using oxygen plasma process. The output spectrum was shifted by 24 nm due to the reaction between the antibody and the virus within 15 min. We show one of the results for the detection for the virus as shown in Fig. 5. When we used the buffer solution without virus on the waveguide, there was no significant change for the output spectrum, which means that there was no phase change of the light in the waveguide. In every experiment, 15–20 min after the applying the aqueous virus solution, the water began to evaporate, thus resulting in no spectral shift finally. Although ELISA is the most common method for detecting the influenza virus, ELISA is time consuming and usually takes prolonged times (~12 h) to obtain the results. On the other hand, our novel optical biosensing system could obtain the result within 15 min.

We are currently attempting to address this issue by fabricating a microflow channel on the MZ waveguide for the virus solution using polydimethylsiloxane (PDMS) to achieve greater reproducibility and prevent evaporation of the virus solution.

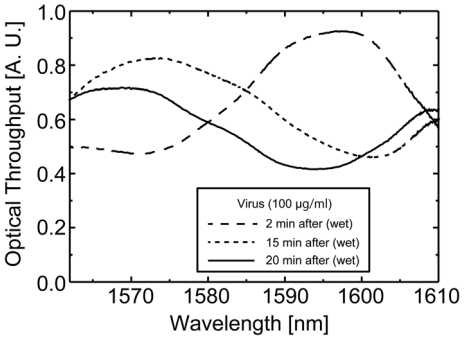

Fig. 5 The time change for the spectrum of the optical output due to application of virus. The spectrum shift was measured 2 (*red*), 15 (*green*), and 20 min (*blue*) after the virus buffer solution was dropped on the MZ waveguide surface

Conclusions

In this study, we developed an optical biosensing system for influenza virus detection. We prepared a carboxyl-modified sol-gel surface, using NHS/EDC as the cross-linker, for antibody immobilization. The results show a significant difference in specific response due to the presence or absence of antigen. The antibody-immobilized sol-gel optical waveguide was used to detect influenza virus binding via spectral shifts. As a result, after treatment with a 100 μg/mL influenza virus solution for 14 min, a peak wavelength shift (~20 nm) was observed in the output light spectrum. To our knowledge, this is the first demonstration of antigen-antibody label-free detection on the sol-gel surface.

In the future, optimization for high sensitivity and reproducibility for the real-time detection are needed, and we planned construction of biosensing system with simplicity and high sensitivity.

References

1. Yohannes, K., Roche, P., Spencer, J., & Hampson, A. (2003). Annual report of the National Influenza Surveillance Scheme, 2002. *Commun Dis Tntell, 27*, 162–172.
2. Montalto, N. J. (2003). An office-based approach to influenza: clinical diagnosis and laboratory testing. *American Family Physician, 67*, 111–118.
3. Poehling, K. A., Griffin, M. R., Dittus, R. S., Tang, Y. W., Holland, K., Li, H., & Edwards, K. M. (2002). Bedside diagnosis of influenza virus infections in hospitalized children. *Pediatrics, 110*, 83–88.
4. Sato, S., Ochiai, H., & Niwayama, S. (1988). Application of the single radical complement fixation test for serodiagnosis of influenza, respiratory syncytial, mumps, adeno type 3, and herpes simplex type 1 virus infections. *Journal of Medical Virology, 24*, 395–404.
5. Enami, Y., Fukuda, T., & Suye, S. (2007). Sol-gel silica planar waveguide doped with green fluorescent protein for in-line biosensors. *Applied Physics Letters, 91*, 203507.
6. Enami, Y., Tsuchiya, K., & Suye, S. (2011). Detection of organophosphorus compound based on a sol-gel silica planar waveguide doped with a green fluorescent protein and an organophosphorus hydrolase. *Applied Physics Letters, 98*, 233503.
7. Enami, Y., Hong, J., Zhang, C., Luo, J., & Jen, A. K. Y. (2011). Optical transmission stability of hybrid sol-gel silica/electrooptic polymer waveguide modulators. *Photonics Technology Letters, 23*, 1508–1510.
8. Enami, Y., DeRose, C. T., Mathine, D., Loychik, C., Greenlee, C., Norwood, R. A., Kim, T. D., Luo, J., Tian, Y., Jen, A. K. Y., & Peyghambarian, N. (2007). Hybrid polymer/sol-gel waveguide modulators with exceptionally large electrooptic coefficients. *Nature Photonics, 3*, 180–185.
9. Suye, S., Enami, Y., (2014) "Optical waveguide biosensors and biosensor system", 2009-264861, Hiroshima University & University of Fukui, Japan Patent No. 5540398, 16 May 2014.
10. Misiakos, K., Raptis, I., Makarona, E., Botsialas, A., Salapatas, A., Oikonomou, P., Psarouli, A., Petrous, P. S., Kakabakos, S. E., Tukkiniemi, K., Sopanen, M., & Jobst, G. (2014). All-silicon monolithic Mach-Zehnder interferometer as a refractive index and bio-chemical sensor. *Optics Express, 22*, 26803–26813.
11. Dante, S., Duval, D., Sepúlveda, B., González-Guerrero, A. B., Sendra, J. R., & Lechuga, L. M. (2012). All-optical phase modulation for integrated interferometric biosensors. *Optics Express, 20*, 7195–7205.
12. Kim, J., Seidler, P., Wan, L. S., & Fill, C. (2009). Formation, structure, and reactivity of amino-terminated organic films on silicon substrates. *Journal of Colloid and Interface Science, 329*, 114–119.
13. Tero, R., Misawa, N., Watanabe, H., Yamamura, S., Nambu, S., Nonogaki, Y., & Urisu, T. (2005). Fabrication of avidin single molecular layer on silica oxide surfaces and formation of tethered lipid bilayer membranes. *e-Journal Surface Science and Nanotechnology, 3*, 237–243.