Detection of organophosphorus compound based on a sol–gel silica planar waveguide doped with a green fluorescent protein and an organophosphorus hydrolase

Y. Enami,[1,a)] K. Tsuchiya,[2] and S. Suye[2]
[1]*Research Institute for Nanodevices and Bio Systems, Hiroshima University, 1–4–2 Kagamiyama Higashi–hiroshima, Hiroshima 739-8527, Japan*
[2]*Graduate School of Engineering, University of Fukui, 3–9–1 Bunkyo Fukui, Fukui 910-8507, Japan*

(Received 23 March 2011; accepted 10 May 2011; published online 7 June 2011)

In this letter, the authors report the real-time detection of an organophosphorus compound using a sol–gel silica planar waveguide doped with a green fluorescent protein and an organophosphorus hydrolase on a yeast-cell surface display. The waveguide was pumped at 488 nm, and it emitted green fluorescence at the far field. The green fluorescent light at 550 nm changed by 50% from the original power 1 min after application of the organophosphorus compound. The results enable the real-time detection of sarin and other biochemicals by using an in-line fiber sensor network. © *2011 American Institute of Physics*. [doi:10.1063/1.3596448]

Si-slot waveguides were used for optical manipulation of biomolecules[1] after living cells were positioned in the relatively narrow slot gap (e.g., slot distance: 100 nm). A silicon nitride-slot waveguide was employed in biosensor devices with the assistance of ring resonators.[2] Compared to the Si-slot waveguide, sol–gel silica planar single-mode (SM) waveguides have a much lower coupling loss (e.g., 0.1 dB) and waveguide loss (e.g., 1–2 dB/cm) (Ref. 3) with SM fibers. In addition, the sol–gel silica waveguide accepts living cells of any size (e.g., more than a few micron) that the slot-waveguide does not accept. The electrical and optical biosensors have been designed to function independently for detecting biomaterials. Electrical and optical connections between the biosensors must be employed for monitoring potentially hazardous biomaterial such as an organophosphorus (OP) compound (e.g., sarin) in the human body. The sol–gel silica waveguide sensor is easily butt-coupled with an optical fiber using UV curable resin. The silica waveguide (2×5 μm) is packaged with a standard method for waveguide devices such as optical modulators.[3] We directly doped a green fluorescent protein (GFP) into a sol–gel silica SM waveguide core,[4] although before, it was difficult to handle living protein inside solid-state waveguide device structures. The GFP was immobilized on the beads surface after it covalently bonded to the carboxyl groups of chemically modified polystyrene beads.[4] The GFP-doped waveguide demonstrated a higher fluorescence power, which was visibly observed at the far field when a blue laser at 488 nm was transmitted through the waveguide core and excited the GFP.[4] The fluorescence power of the GFP changed according to the pH variation near the GFP.[5] When a pH 4.0 buffer solution was applied to the waveguide core, green fluorescence power out of the waveguide was reduced by a factor of 10 within 3 min; ultimately, the green fluorescence spot at the far field visibly disappeared.[4] We also showed that the waveguide emitted green fluorescence out of the waveguide at the far field for more than one month, which was a longer lifetime in the sol–gel silica core than in the standard optimized buffer solution. An OP hydrolase (OPH) and enhanced GFP (EGFP) yeast cell-surface display worked as a biophotonic sensor for detecting the OP compound because the chemical reaction between OP and OPH varies pH, which changes the green fluorescence power. Moreover, when the OPH and GFP were displayed on the yeast cell surface using DNA manipulation, the chemical reaction between OP and OPH improved significantly, which directly affected the change in pH at a nanoscale volume surrounding GFP, resulting in a larger change in green fluorescence power.[6] Therefore, when the OPH and GFP displayed on the yeast cell surface were doped in the sol–gel silica waveguide core and pumped at 488 nm, the waveguide detected the OP compound by the change in green fluorescence power. This study demonstrates the detection of the OP compound by the change in the green fluorescence power in a sol–gel silica waveguide doped with the GFP and OPH on a yeast cell-surface display.

The method for immobilizing EGFP and OPH on a yeast cell surface was explained in detail in the previous reports.[6,7] The EGFP and OPH immobilized on a yeast cell surface were doped in a sol–gel core (4 μm wide, 4 μm high, 5 mm long). A SM waveguide biophotonic device was fabricated, as shown in Fig. 1 using the method demonstrated for the sol–gel waveguide doped with the GFP model cell.[4] The process of the sol–gel silica waveguide fabrication was described in detail in a previous report.[3] Sol–gel solutions with molar ratios of 85% methacryloyloxy propyltrimethoxylane (MAPTMS) to 15% index modifier [zirconium (IV)-*n*-propoxide (ZrPO)] and 88% MAPTMS to 12% ZrPO were prepared for the sol–gel core and cladding, respectively. An 8 μm thick sol–gel under cladding was spin coated over a SiO_2 (6 μm thick)/Si substrate and baked at 150 °C for 1 h, as shown in Fig. 1(a). The 4 μm thick sol–gel side cladding layer was coated on top of the under cladding and wet etched in isopropanol after UV light (mercury i-line: 9 mW/cm^2) in a mask aligner was radiated over the side cladding layer through a photolithographic mask for defining the waveguide side cladding structure. The nonirradiated part of sol–gel side cladding layer was removed from isopropanol and baked at 80 °C for 24 h [Fig. 1(b)].

a)Electronic mail: yenami@hiroshima-u.ac.jp.

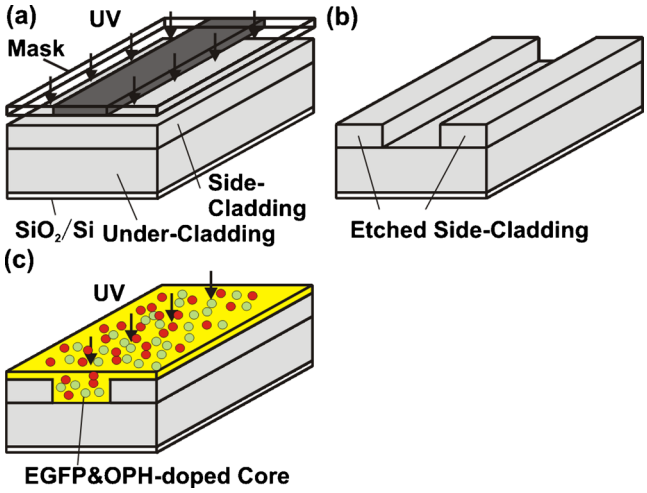

FIG. 1. (Color online) Fabrication process for the EGFP- and OPH-doped waveguide device for detecting the OP compound. (a) The side cladding layer coated on the under cladding/SiO_2/Si layer is radiated using UV light though the photolithographic mask for patterning of side cladding. (b) The EGFP-doped waveguide device is developed in isopropanol to remove the nonirradiated part. (c) The EGFP- and OPH-doped sol–gel core solution is spin coated between the sidewalls of the side cladding. UV is radiated to partially promote the silica network in the sol–gel core. After UV radiation, the EGFP-doped sol–gel waveguide device is cleaved to couple light from the SM fiber.

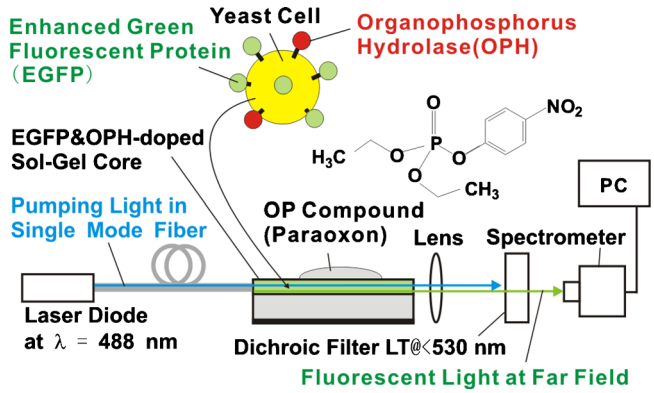

FIG. 2. (Color online) Schematic of the experimental setup. EGFP and OPH immobilized on the yeast cell surface are directly doped in the sol–gel silica core. The pump laser is coupled from the SM fiber into the waveguide device. Fluorescent light from the device is collected using a 20× microscope objective lens and focused on the spectrometer though the dichroic beam splitter to filter the pump light. The fluorescence spectrum is analyzed on a computer. Paraoxon, an OP compound, is mixed with 10% methanol/DI water and applied to the waveguide.

Glycerol (0.3%–0.7% volume) was added to the sol–gel core solution for improving the penetration of the OP solution[8] into the sol–gel silica core. We optimized the amount of glycerol in the sol–gel silica core after observing that the OP solution penetrated into the fabricated sol–gel silica waveguide within 10 min. The EGFP and OPH immobilized on the yeast cell in the buffer solution were mixed with the sol–gel core solution at the 2.56 fM doping level EGFP. The sol–gel core solution was coated on the sol–gel cladding etched on the substrate and filled in the trench between the sol–gel side cladding, as shown in Fig. 1(c). The reversed ridge core was radiated with UV light in the mask aligner for 10–15 min without postbaking. This process accelerated the hydrolysis of the sol–gel silica core and partially cross-linked the sol–gel silica network in the core without killing the living EGFP and yeast cells, which are sensitive to high temperatures. It also maintained an optimized porous size in the silica. The OP solution can penetrate the optimized sol–gel silica core within 10 min. The immobilized EGFP and OPH on the yeast cell surface also had an optimized distance in the nanosized volume to react with the OP compound and change the pH, which resulted in the change in the green fluorescence power.[6] The fabricated waveguide was cleaved along the Si crystal axis to have the straight end facet with a coupling loss of 1–2 dB with SM fiber (mode field diameter of 4.2 μm) that can be reduced further with optimization of the refractive index difference between the core and cladding. The refractive index was controlled (e.g., within 0.0001) by varying the doping level of ZrPO. The waveguide propagating loss was 1 dB per 5 mm at 515 nm.

A laser at 488 nm was propagated from a diode through the SM fiber and butt-coupled into the EGFP- and OPH-doped waveguide. The green fluorescent light emitted from the waveguide was collected and focused by a 20× microscope objective lens on a spectrometer with a 1.02 nm wavelength resolution through a dichroic filter ($T \sim 10^{-5}$ at 422–530 nm) for removing the 488 nm pumping light. The fluorescent light received by the spectrometer was digitally processed and transferred to a computer for analyzing the fluorescence spectrum, which monitors the change in the fluorescence power at 550, 600, 650, and 700 nm. The fluorescence spectrum showed the center wavelength of 550 nm and the fluorescence wavelength up to 800 nm, as shown in Fig. 3. The dichroic filter cut the pumping light at 488 nm and the fluorescence light lower than 530 nm. As an OP compound, 20 mM paraoxon was mixed with 10% methanol in distilled (DI) water (10% methanol/DI water). While monitoring the fluorescence spectrum in the waveguide, 4 μl of paraoxon (chemical structure in Fig. 2) solution was dropped on the waveguide core.

When the paraoxon penetrated the sol–gel core, the fluorescence power reduced at each wavelength. The change in the fluorescence power at 550 nm is shown as an example in Fig. 4. After 20 mM paraoxon was applied to the waveguide, the fluorescence power was reduced by 50% in 1 min and to

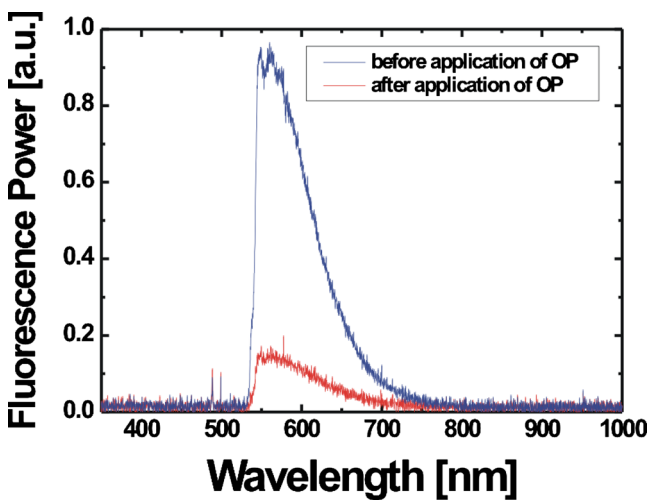

FIG. 3. (Color online) Fluorescence spectrum at the far field of the waveguide. Upper: before application of OP compound. Lower: after application of OP compound.

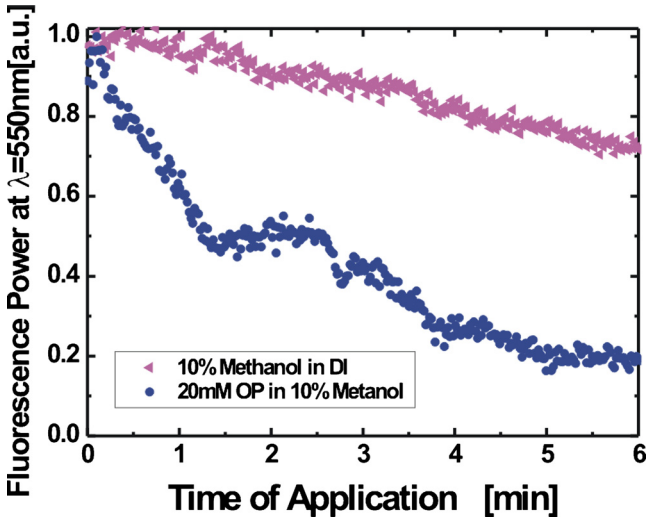

FIG. 4. (Color online) Change in the fluorescence power after application of 20 mM paraoxon mixed with 10% methanol/DI water. Solid triangles represent output power after application of 10% methanol/DI water. Solid circles represent output power after application of 20 mM OP mixed with 10% methanol/DI water.

20% of the original fluorescence power in 6 min on account of the photobleach effect of the EGFP. To confirm if the reduction was caused by the OP compound, 4 μl of 10% methanol/DI water was applied to another waveguide on the same substrate. The fluorescence power was reproduced and linearly reduced to approximately 70% of the original value in 6 min. From these results, we concluded that the change in the fluorescence power at each wavelength was caused solely by 20 mM OP compound in 10% methanol/DI water. When the mixing amount of glycerol is less than 0.1% volume in the sol–gel silica core solution, the reduction in the fluorescence power was similar to the results obtained from the 10% methanol/DI water applications. On the other hand, when the mixing amount of glycerol is more than 1% volume in the sol–gel silica core solution, the thin-film quality of the spin-coated core on the cladding degraded and developed visible spots on the film, which did not work as a low-loss waveguide.

In summary, we detected the OP compound within 1 min of the change in the fluorescence power for the waveguide doped with EGFP and OPH immobilized on the yeast cell surface. We designed the waveguide OP sensors to be butt-coupled and connected them with the SM fiber. The serial connection of the sensors through the fibers enabled a compact, distributed biophotonic sensor network without any electronics in the sensing parts. There was no obvious decrease in fluorescence response of OPH for OP compound in the sol–gel silica thin film at room temperature or 4 °C for one month. However, when the waveguide device was heated at more than 50 °C for several hours, the fluorescence was decreased due to the decomposition of the protein. The device would be operated for more than one month at lower temperature range. OPH has broad substrate specificity and is able to hydrolyze a number of OP pesticides such as paraoxon, parathion, coumaphos, diazinon, dursban, methyl parathion, etc., and chemical warfare agents, sarin and soman. We believe that we can easily extend this system in detection other OPH variants. Sarin can spread very fast, and the reaction with OPH in the sol–gel silica is faster than with paraoxon. The sol–gel silica film with GFP and OPH on the yeast detected 10 ppm of paraoxon under a fluorescent microscope. The waveguide device would detect sarin with similar sensitivity to paraoxon. Therefore, the waveguide biophotonic sensors could be applied for sarin detection among other special safety considerations for such an experiment.

This work was supported by a Grant-in-Aid for Scientific Research (A) from the Ministry of Education Culture, Sports, Science, and Technology (Grant No. 09020790), Japan, International Collaborative Grant by National Institute of Information and Communications Technology (Grant No. 09150703), Japan, A-STEP by Japan Science and Technology Agency (Grant No. 09158836).

[1]A. H. J. Yang, S. D. Moore, B. S. Schmidt, M. Klug, M. Lipson, and D. Erichson, Nature (London) **457**, 71 (2009).
[2]C. A. Barrios, M. J. Bauls, V. Gonzalez-Pedro, K. B. Gylfason, B. Sanchez, A. Griol, A. Maquieira, H. Sohlstrom, M. Holgado, and R. Casquel, Opt. Lett. **33**, 708 (2008).
[3]Y. Enami, C. T. DeRose, D. Mathine, C. Lochik, C. Greenlee, R. A. Norwood, T. D. Kim, J. Luo, Y. Tian, A. K.-Y. Jen, and N. Peyghambarian, Nat. Photonics **1**, 180 (2007).
[4]Y. Enami, T. Fukuda, and S. Suye, Appl. Phys. Lett. **91**, 203507 (2007).
[5]T. Fukuda, S. Shiraga, M. Kato, S. Suye, and M. Ueda, Biotechnol. Prog. **22**, 944 (2006).
[6]K. Takayama, S. Suye, K. Kuroda, M. Ueda, T. Kitaguchi, K. Tsuchiama, T. Fukuda, W. Chen, and A. Mulchandani, Biotechnol. Prog. **22**, 939 (2006).
[7]T. Fukuda, K. Tsuchiyama, H. Makishima, K. Takayama, A. Mulchandani, K. Kuroda, M. Ueda, and S. Suye, Biotechnol. Lett. **32**, 655 (2010).
[8]X. Ma, H. Sun, and P. Yu, J. Mater. Sci. **43**, 887 (2008).