IEICE TRANS. ELECTRON., VOL.E100–C, NO.2 FEBRUARY 2017

INVITED PAPER *Special Section on Fabrication Technologies Supporting the Photonic/Nanostructure Devices*

Label-Free Optical Detection of Fibrinogen in Visible Region Using Nanoimprint Lithography-Based Two-Dimensional Photonic Crystal

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SUMMARY For the future medical diagnostics, high-sensitive, rapid, and cost effective biosensors to detect the biomarkers have been desired. In this study, the polymer-based two-dimensional photonic crystal (2D-PC) was fabricated using nanoimprint lithography (NIL) for biosensing application. In addition, for biosensing application, label-free detection of fibrinogen which is a biomarker to diagnose the chronic obstructive pulmonary disease (COPD) could be achieved using antigen-antibody reaction high-sensitively (detection limit: pg/ml order) and rapidly. Using this polymer-based 2D-PC, optical biosensor can be developed cost effectively. Furthermore, by using polymer as a base material for fabrication of 2D-PC, label-free detection of antigen-antibody reaction can be performed in visible region.

key words: *photonic crystal, biosensor, polymer, nanoimprint lithography*

1. Introduction

High-sensitive, rapid and cost effective biosensors for medical diagnostics are increasing importance to healthcare and medicine. To diagnose the diseases such as cancers, infection diseases, and neurodegenerative disorders, biosensors can detect the biomarkers such as proteins and DNAs which is related to these diseases using several detection principles based on electrochemistry [1] and optics [2]. In the past two decades, a multitude of biosensors using different detection principles had been reported for detection of biomarkers high sensitively [3]. Because, to diagnose the diseases, low concentration of biomarkers must to be determined in the body fluids for prevention of diseases. From the previous report, many biosensors had achieved to determine the low concentration of biomarkers.

Especially, during recent years, nanooptical biosensors based on photonic crystal (PC) [4], toroidal cavity [5], noble metal nanoparticles [6] achieved to develop the high sensitive biosensors. These biosensors enable to detect the optical characteristics change due to the surrounding refractive index change which attributed by the specific interaction between biomarkers and biorecognition elements such as antibody and probe DNA. In addition, optical characteristics will be drastically changed by the fractional refractive index change. Hence, nanooptical biosensors can detect the biomarkers without additional sophisticated liquid handling procedures such as a labeling procedure using fluorescent dyes and enzymes [7].

However, to determine the concentration of biomarkers in body fluids using previously developed nanooptical biosensors has several disadvantages in cost-effectiveness. For fabrication of these nanooptical biosensors require the high cost fabrication instruments and methods such as electron beam lithography (EBL) and reactive ion etching (RIE), and focused ion beam (FIB) [8]. In addition, for detection of biomarkers require the high cost measurement equipment such as spectrum analyzer with high wavelength resolution and infrared laser light source.

To improve the above-mentioned disadvantages, we have been developing polymer-based nanooptical biosensor which act in visible region. To fabricate the polymer-based nanooptical biosensor cost effectively and rapidly, we have been using the nanoimprint lithography (NIL) [9]–[13]. Using NIL, nanooptical biosensor can be fabricated with high reproducibility and cost effectively. Based on polymer-based nanooptical biosensor, we could have achieved to fabricate the polymer-based two-dimensional photonic crystal (2D-PC). In addition, using this polymer-based 2D-PC, high-sensitive label-free biosensor which detect the biomarkers (influenza virus, insulin, and urokinase prasminogen activator (uPA)) as surrounding refractive index change by the antigen-antibody reaction and DNA hybridization in visible region was successfully developed. From our previous achievement, fabrication techniques of nanooptical devices using NIL have been called as “printable photonics”.

From these backgrounds, in this study, nanooptical biosensor for detection of fibrinogen which is a biomarker to diagnose the chronic obstructive pulmonary disease (COPD) was developed using polymer (cyclo-olefin polymer (COP))-based 2D-PC [14]. COPD is a common lung disease and leading cause of death worldwide [15]. Hence, by detection of fibrinogen high sensitively using simple optical setup, number of patients can be reduced. In this study, sensing performance of polymer-based 2D-PC for fibrinogen using antigen-antibody reaction was performed.

Manuscript received June 15, 2016.
Manuscript revised September 21, 2016.
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DOI: 10.1587/transele.E100.C.166

2. Experiments

2.1 Reagents

For the determination of fibrinogen using polymer-based 2D-PC, goat anti-human fibrinogen antibody (55036) was purchased from MP Biomedicals (Tokyo, Japan). And human fibrinogen (341576) were purchased from Merck Millipore (Darmstadt, Germany). To immobilize the anti-human fibrinogen antibody onto the 2D-PC surface, 3-aminopropyltriethoxysilane (γ-APTES) (S330) (JNC (Tokyo, Japan)) and 25% (v/v) glutaraldehyde (GA) (079-00533, WAKO pure chemicals (Osaka, Japan)) were used. For blocking, ethanolamine hydrochloride (E61333) were purchased from Sigma-Aldrich Japan K. K. (Tokyo, Japan). In addition, ultra-pure water (18.2 MΩ-cm) from Sartorius Stedim Biotech (Aubagne, Cedex, France) was used in all sample preparations.

2.2 Apparatus

For the fabrication of 2D-PC, NIL was performed using a nanoimprint apparatus (X-300, SCIVAX Corp., Kanagawa, Japan). Evaluation of optical characteristics was done using the handy type spectrophotometer (USB-4000-UV-VIS, wavelength range: 200–1100 nm) with a tungsten halogen light source (LS-1, wavelength range: 360–2000 nm), which were purchased from Ocean Optics (Dunedin, USA). The bifurcated fiber bundle (BFY200HS02, fiber core diameter: 200 μm, wavelength range: 300–1200 nm) was purchased from Thorlabs (Tokyo, Japan).

For observation of surface construction of the 2D-PC, scanning electron microscopy (SEM) was used from Keyence (VE-9800, Osaka, Japan).

2.3 Experimental Procedure for Detection of Fibrinogen Using Polymer-Based 2D-PC

Experimental procedure for detection of fibrinogen using polymer-based 2D-PC was shown in Fig. 1. When the white light was irradiated to the polymer-based 2D-PC, our polymer-based 2D-PC exhibit the diffraction spectrum which has a specific peak wavelength based on Bragg's law. In addition, for detection of antigen-antibody reaction, anti-human fibrinogen antibody was immobilized onto the polymer 2D-PC surface, and the specific antibody-antigen reaction between fibrinogen (antigen) and antibody were occurred by introduction of sample solution. By these specific reaction, the surrounding refractive index will be increased which depend on the fibrinogen concentrations. These increment of surrounding refractive index due to the antigen-antibody reaction, the diffraction peak intensity-based on Bragg's law will be decreased based on Fresnel equations [9], [10], [13]. From these experimental procedure, fibrinogen concentration can be determined as a diffraction peak intensity change.

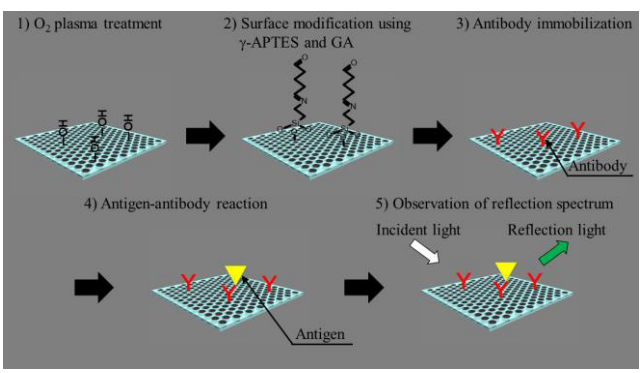


Fig. 1 Experimental procedure for detection of antigen-antibody reaction using polymer-based 2D-PC.

Using Bragg's law, if the refractive index change was occurred, diffraction peak will be shifted. However, the amount of refractive index change due to the antigen-antibody reaction is negligible. Hence, the spectrum analyzer with high wavelength resolution are required. However, by using this detection principle, fractional surrounding refractive index change can be detected by the diffraction peak intensity change of 2D-PC.

2.4 Fabrication of Polymer-Based 2D-PC Using Printable Photonic Technology

In this study, polymer-based 2D-PC which have triangular configured pillar array (lattice constant: 460 nm, pillar diameter: 230 nm, pillar height: 200 nm) was fabricated using nanoimprint apparatus (X-300, SCIVAX corporation) onto COP film (thickness: 100 μm, refractive index: 1.53, transparency: 92% at 400~800 nm) by thermal NIL (welding pressure: 10 MPa, temperature: 180°C, film diameter: 4 inch). By using this configuration and sizes, structural color can be observed by naked eyes. In addition, from the structural color, fabrication accuracy of 2D-PC can be evaluated easily. After the fabrication of polymer-based 2D-PC, antibody immobilization was carried out onto the polymer-based 2D-PC surface.

2.5 Antibody Immobilization

The anti-human fibrinogen antibody was immobilized onto polymer-based 2D-PC surface. For immobilization of antibody, O_2 plasma treatment (gas flow rate: 15–26 sccm, power: 80 W, 25 min) using an O_2 plasma cleaner (CUTE-1MP/R (Femto Science, Inc., Gyeonggi-Do, Korea)) was carried out for introduction of hydroxy-group onto 2D-PC surface. After the O_2 plasma treatment, anti-human fibrinogen antibody was immobilized (1 μg/ml, 1h at room temperature) via a surface modification using γ-APTES (0.1% (v/v)) (1 h, room temperature) and GA (0.5% (v/v)) (1 h, room temperature). And then, after the immobilization of antibody, ethanolamine solution (100 mM) (1 h, room temperature) was introduced for blocking [16]. From these anti-

body immobilization procedure, detection of fibrinogen was carried out.

2.6 Label-Free Detection of Antigen-Antibody Reaction Using Antibody Immobilized Polymer-Based 2D-PC

For detection of fibrinogen using antigen-antibody reaction, different concentration of fibrinogen solutions (1 pg/ml~1 μg/ml) were introduced onto the antibody immobilized polymer-based 2D-PC for 1h at room temperature for antigen-antibody reaction. After the antigen-antibody reaction, the excess amount of antigens was removed by washing and dry up procedure using phosphate buffer (10 mM, pH 7.4) for three times.

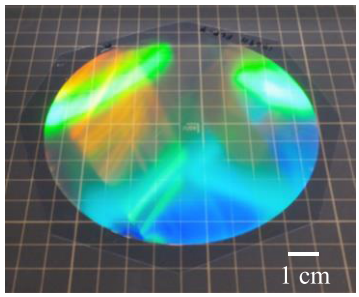
For evaluation of optical characteristics change of polymer-based 2D-PC by antigen-antibody reaction, diffraction spectrum was monitored using handy type spectrophotometer. The white light from tungsten-halogen lamp were irradiated from perpendicular direction (distance: 30 μm), and the diffraction light from the polymer-based 2D-PC was monitored (400~800 nm) using a bifurcated fiber bundle (numerical aperture: 0.2). Using this simple optical characterization setup, the diffraction peak intensity changes due to the surrounding refractive index change-based on antigen-antibody reaction was determined.

3. Results and Discussions

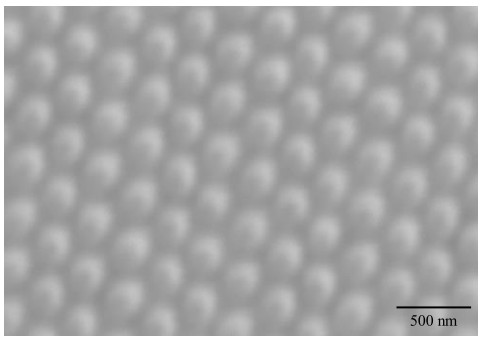
3.1 Fabrication of Polymer-Based 2D-PC Using Printable Photonics Technology

Photograph of polymer-based 2D-PC was shown in Fig. 2 (a). By using printable photonics technology, polymer-based 2D-PC, structural color by the Bragg's law could be observed with high reproducibility. In addition, by the SEM observation, periodic nanostructure could be observed. However, from the SEM image, pillar diameter was slightly increased. The increment of pillar size diameter attributed by the demolding process. In addition, using printable photonics technology, we could have fabricated 2D-PC using different base materials such as metals [17], [18].

Furthermore, from the previous our study, height and periodicity of pillar will be affected to the diffraction peak intensity [9], [10], [13]. Based on our previous study, height of pillar was approximately 200 nm. In addition, from the SEM observation, periodicity of pillars is lower than the previously reported PC-based biosensors [4], [5], [8]. These are affected by NIL procedure such as swelling or shrinking of base materials, demolding (surface treatment of mold release agent and demolding direction), and fabrication accuracy of mold. To fabricate the highly accurate polymer-based 2D-PC, improvement of these factors are required. However, for biosensing application, in this study, white light was irradiated to the wide area of 2D-PC surface via a fiber (fiber core diameter: 200 μm). Hence, the variability of configuration, size and periodicity of pillars will be averaged.



(a)



(b)

Fig. 2 (a) Photograph of polymer-based 2D-PC. (b) SEM image of polymer-based 2D-PC surface.

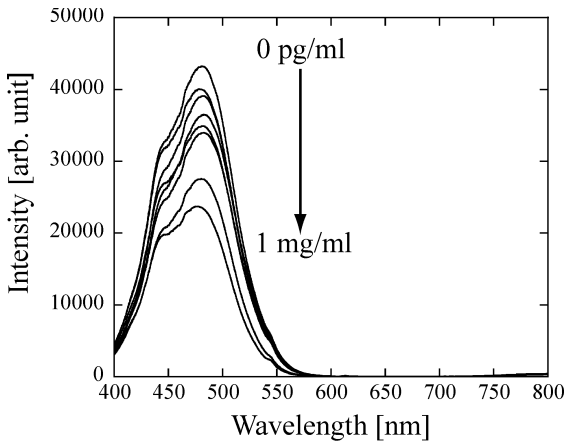


Fig. 3 Diffraction spectrum change by antigen-antibody reaction using polymer-based 2D-PC

3.2 Detection of Antigen-Antibody Reaction Using Antibody Immobilized Polymer-Based 2D-PhC

The diffraction spectrum change by antigen-antibody reaction was shown in Fig. 3. Using our polymer-based 2D-PC has a diffraction peak at 481.7 nm based on Bragg-diffraction. Based on Bragg's law, diffraction peak wavelength is shifted toward longer wavelengths from the estimated peak wavelength (approximately 460 nm) due to the transferred pillar size change.

In addition, when the introduction of 1 μg/ml of fibrinogen solution, the diffraction peak intensity was dras-

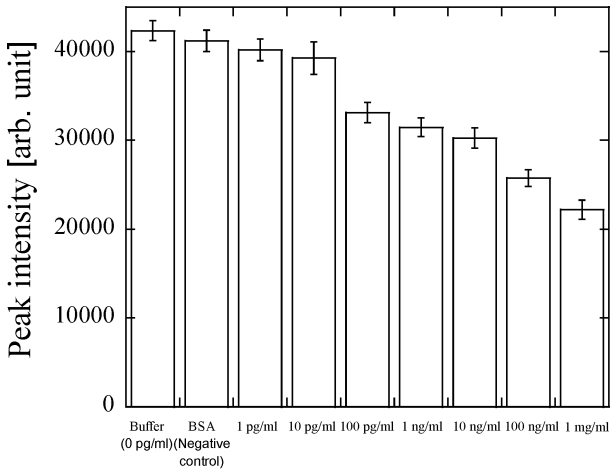


Fig. 4 Antigen concentration dependency for diffraction peak intensity change.

tically decreased by the increment of surrounding refractive index. On the other hand, as a negative control, bovine serum albumin (BSA) solution (1 μg/ml) was introduced onto the antibody immobilized polymer-based 2D-PC, diffraction peak intensity change could not be observed (data not shown). Hence, the antibody-immobilized polymer-based 2D-PC recognize the target molecules specifically.

In addition, antigen concentration dependency for diffraction peak intensity change was shown in Fig. 4. By introducing of different concentration of antigen solutions, diffraction peak intensities were changed which depend on the antigen concentrations. Furthermore, from the concentration dependency. We found that the polymer-based 2D-PC enable to detect the fibrinogen down to 100 pg/ml. From the previous report, D. Valvi *et al.* reported that the mean fibrinogen concentration in blood serum of COPD patients was approximately 4.0 mg/ml. From these results, this polymer-based 2D-PC has an enough sensitivity for detection of fibrinogen [19].

However, from the previous report of nanooptical biosensors [4], detection limit of this 2D-PC is not enough for detection of low concentration of target molecules such as cancer makers. Hence, to realize the high-sensitive biosensor using 2D-PC, several improvements such as refractive index of base materials and design are required.

4. Conclusions

In this study, fabrication of polymer-based 2D-PC for biosensor application based on printable photonics technology was succeeded. For medical diagnostics, to determine the fibrinogen concentration in blood serum, enzyme-linked immunosorbent assay (ELISA) have been widely used. However, ELISA require sophisticated liquid handling, long assay time (3~4 h) and enzyme conjugated secondary antibody. On the other hand, using our polymer-based 2D-PC, detection of fibrinogen can be performed rapidly (1 h) and cost effectively. From these experimental results, we have been developing the higher sensitive, and cost effective biosensor using printable photonics technology-based nanooptical devices.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (B) (15H03009) from the Japanese Ministry of Education, Culture, Science, Sports and Technology (MEXT), Ministry of Health, Labor and Welfare Grants-in-aid for Scientific Research and A-STEP (Adaptable & Seamless Technology Transfer Program through Target-driven R&D) from the Japan Science and Technology (JST).

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