Specific Detection of Antibiotics by Silicon-on-Chip Photonic Crystal Biosensor Arrays

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***Abstract*— Photonic crystal (PC) microcavities formed by 2-D air holes on silicon-on-insulator substrates were utilized as biosensors to detect antibiotics with high specificity. The antibiotic, gentamicin, with molecular weight of only 478 g/mol, was successfully detected via its binding to corresponding antibody pre-immobilized on the sensor surface. Detection range (in concentration) was extended by combining different types of PC biosensors on a single silicon chip, covering from 0.1 nM to 1 μM. Specificity of detection was also verified on the studied antibiotic.**

***Index Terms*— Photonic crystals, silicon photonics, optical biosensors, microcavities, antibiotics.**

I. Introduction

THERAPEUTIC drug monitoring helps maximize the therapeutic effect while minimizing adverse effects [1]. Conventional methods have been developed for the measurement of antibiotics, including immunoassays, spectrophotometry, high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) [2]–[5]. These methods are usually expensive and time-consuming. Innovative detection methods with high sensitivity and low cost are needed. Over the last two decades, on-chip silicon photonic biosensors have become a fast-growing research topic owing to the advantages of high sensitivity, high throughput, and no need for labeling. Various devices, including microring resonators [6]–[9], microdisks [10], silicon nanowires [11], nanoporous silicon waveguides [12], Bragg gratings [13], [14], subwavelength grating waveguides [15], [16], 1D and 2D photonic crystal (PC) microcavities [17]–[22], have been demonstrated. The 2D PC microcavity sensor is unique in its slow light enhanced sensitivity within a miniature footprint and flexibility in structural design to engineer its sensing properties.

Manuscript received June 9, 2017; revised July 29, 2017; accepted July 30, 2017. Date of publication August 2, 2017; date of current version August 22, 2017. This work was supported in part by Army (USAMRAA) under Contract W81XWH-14-C-0029 and in part by the AFOSR MURI Program under Grant FA9550-08-1-0394. The associate editor coordinating the review of this paper and approving it for publication was Dr. Ioannis Raptis. *(Corresponding author: Hai Yan.)*

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Digital Object Identifier 10.1109/JSEN.2017.2734885

We have previously reported several PC microcavity biosensor structures which consist of a PC microcavity side-coupled to a PC waveguide. The *Ln* PC microcavity is formed by a row of n missing holes in the Γ-K direction of a hexagonal PC lattice of air holes in silicon (see Fig.1). PC microcavities, such as L13, L21, L55 and L13 with nanoholes, have been reported, offering different minimum detection limits from as low as 1 fM to over 1 μM for detecting biotin-avidin binding [18], [23]–[25]. In this paper, we report a PC microcavity biosensor array by combining different L-type PC microcavity sensors using a multimode interferometer (MMI) power splitter and demonstrate the detection of antibiotics over a concentration range covering 5 orders of magnitude (from 0.1 nM to 1 μM). We explored the sensing of gentamicin antibiotics (molecular weight 478 g/mol) by detecting the binding of antibiotics (target) to anti-gentamicin antibody (probe) that was pre-immobilized on the optical sensor surface. The high sensitivity and label-free detection of integrated photonic sensors with PC microcavities makes possible the detection of these small biomolecules. Detection range in concentration was extended by combining different types of PC biosensors on a single silicon sensor chip. Specificity of detection was also verified on the studied antibiotic.

II. Device Design

In a PC microcavity biosensor, the detection limit and sensitivity are determined by factors including quality factor (Q), group index (n_g), fill fraction (f), etc. Increased quality factor results in narrower linewidth of the resonance and enhanced interaction time between the optical mode and the analyte. Therefore, the sensitivity is increased and the detection limit ($D = \lambda/QS$) becomes lower. On the other hand, the slow light effect (high n_g) in the coupling waveguide, which enhances light-matter interaction in the cavity, brings unique advantage for high sensitivity sensing. From L3 to L13 and L55 PC microcavities, with increasing cavity length, the sensors possess improved sensitivity and reduced detection limit owing to increasingly high Q and n_g [18], [23]–[25].

Another critical design consideration is the analyte overlap with cavity modes, characterized by fill fraction (f) [24]:

$$f = \frac{\left\langle \vec{E}_m \left| \varepsilon \right| \vec{E}_m \right\rangle_{V_{liquid}}}{\left\langle \vec{E}_m \left| \varepsilon \right| \vec{E}_m \right\rangle_{V_{liquid+dielectric}}} \tag{1}$$

where $\vec{E}_m$ denotes the electric field of the mth mode and ε is the dielectric constant. The sensitivity of the microcavity

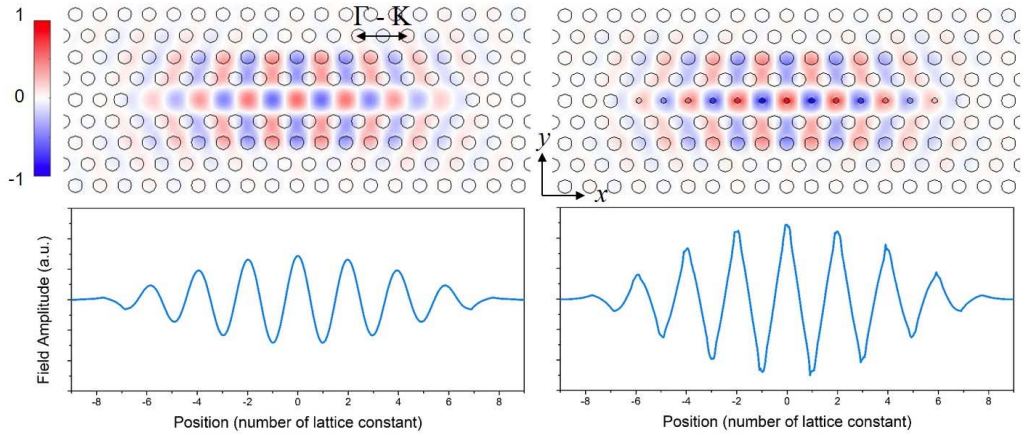

Fig. 1. Mode profiles (E_y) of L13 and L13 with nanoholes PC microcavities.

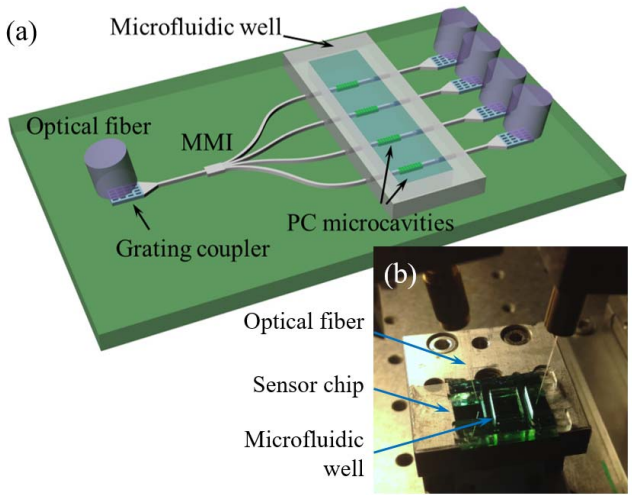

Fig. 2. (a) Schematic of the PC microcavity biosensor array. (b) The sensor chip on a fiber coupling test stage.

sensor is proportional to the fill fraction [24]. The L13 with nanoholes microcavity has small holes inside the cavity which results in significantly increased fill faction ($f = 0.14$ for L13 with nanoholes, an increase of 40% from $f = 0.10$ for L13 cavity, see Fig. 1). We were able to achieve a high sensitivity (112 nm/RIU) and low detection limit (1 fM) from the L13 with nanoholes biosensors [24].

Considering the above factors that affect the detection range and minimum detection limits of PC biosensors, four distinct types of 2D PC sensors with different geometries were chosen to achieve wide dynamic range detection in the drug molecule sensing. They are L3, L13, L55 and L13 with nanoholes. They were combined in a 1 × 4 multimode interferometer (MMI) power splitter to allow for simultaneous testing of all microcavities in the same measurement. A schematic of the system is shown in Fig. 2(a). Grating couplers were used to couple continuous wave (CW) light in and out of the waveguide devices. Samples containing biomolecules were applied on the PC microcavities inside a microfluidic well made of polydimethylsiloxane (PDMS).

The sensor chip was fabricated on a silicon-on-insulator (SOI) wafer with 250 nm thick silicon device layer. The device pattern was defined by e-beam lithography and transferred to the silicon layer by reactive ion etching. The lattice constant of the PC is designed as $a = 392.5$ nm and the air holes have a radius $r = 108$ nm as in [24]. In L13 PC microcavities with nanoholes device, the nanoholes have a radius of 0.4 r = 43 nm. The grating couplers were designed following the principles in [23] with grating period of 690 nm and duty cycle of 50%. The length and width of the MMI are 123μm and 16μm, respectively. All input and output waveguides at the MMI are 2.5μm wide. The output waveguides are separated by 1.5μm. Fig. 3(a) shows a stitched microscope image of the MMI and the four PC sensors on the four optical paths. The insets in Fig. 3(b)-(e) show the images of the individual PC sensors observed under scanning electron microscope (SEM).

III. Biosensing Test

The sensor chip needs chemical treatment before the biosensing experiment in order to immobilize probe antibodies covalently. After the sensor chip was fabricated, thermal oxidation is done at 950 °C for 5 min to grow about 5 nm silicon dioxide on the sensor surface. The oxide layer serves as the base for subsequent chemical treatment. The chip was next silanized with 2% (v/v) (3-aminopropyl)triethoxysilane (APTES) in toluene. After a thorough clean with toluene, the chip was further treated with 2.5% (v/v) glutaraldehyde in phosphate buffer saline (PBS) to provide an aldehyde group linker that is able to immobilize probe antibodies covalently [26]. After that, a microfluidic well (7mm long × 5mm wide and 2mm high) made of Polydimethylsiloxane (PDMS) was mounted on the sensor chip (see Fig. 2(b)). The following reaction and test were done in the microfluidic well in a static condition. The probe antibody was immobilized on the sensor surface by dispensing anti-gentamicin antibodies (1mM in PBS) in the microfluidic well and incubating for 60 min after which the device is washed thoroughly with PBS to remove any unbound antibodies. Before target binding test, 1% bovine serum

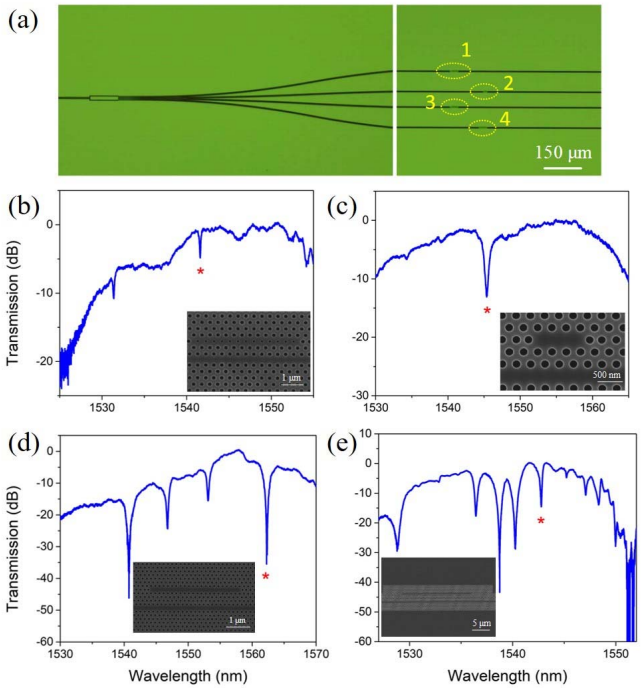

Fig. 3. (a) Stitched microscope image showing the MMI coupler and four PC sensors on the four channels. (b)-(e) Transmission spectra and SEM images of PC microcavities side-coupled to PC waveguides: (b) L13 with nanoholes, (c) L3, (c) L13, (d) L55. Red star markers show the resonance dip used for biosensing test.

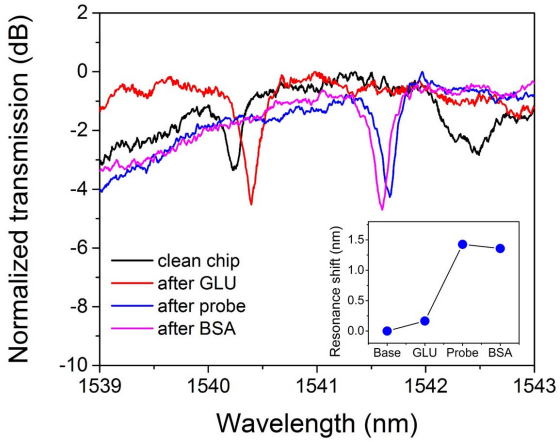

Fig. 4. Transmission spectra of a L13 with nanoholes before and after functionalization. Inset shows the resonance shift at each step. GLU: glutaraldehyde; probe: anti-gentamicin antibody; BSA: bovine serum albumin.

albumin (BSA) was applied to block any binding sites that have not been covered by probe antibodies. After a thorough wash in PBS, the device was ready for antibiotics sensing test.

The transmission spectra of the PC sensors were obtained using a broadband amplified spontaneous emission (ASE) source (1510 nm-1630 nm) and an optical spectrum analyzer (OSA). The measured transmission spectra for the four PC sensors are shown in Fig. 3(b)-(e).

Figure 4 shows the transmission spectra of a L13 with nanoholes PC biosensor before and after the above functionalization steps. All measurements are done with PBS as background. A red shift of 0.16 nm and 1.43 nm after applying glutaraldehyde and probe (anti-gentamicin antibody) are indications of surface mass accumulation with chemical films (APTES and glutaraldehyde) and immobilized probes. The slight blue shift after applying BSA is probably due to insufficient wash after probe incubation while some probe molecules were washed away at BSA step. After BSA incubation, the chip was washed thoroughly with PBS to create a baseline for target binding test.

All target antibiotics were spiked in 1% BSA in PBS. The sensors were incubated in target solutions for 40 minutes at room temperature and then washed with PBS. Transmission spectra of the PC sensors were measured after each step in the same PBS environment in order to compare the resonance wavelength shifts. The experiments were divided into two parts: (a) sensor array demonstration and (b) specificity verification.

A. Sensor Array Demonstration

Sensor chips with all four types of PC sensors, L3, L13, L55 and L13 with nanoholes combined by a 1×4 MMI were used. Anti-gentamicin antibody at high concentration of 1mM was used as the probe to create enough binding sites on the sensor surface. Gentamicin concentrations from 1 nM to 10 μM with a step of an order of magnitude were used as the target to be detected. The resonance shift for each sensor was measured and the corresponding concentration recorded.

B. Specificity Verification

In this part, sensor chips with only L13 PC microcavity sensors were used. The chips were activated and immobilized with anti-gentamicin antibody. The chip was then tested with all three antibiotics, all at a concentration of 1 mM. The resonance shift for each antibiotic was recorded.

IV. Results and Discussion

A wide concentration range (5 orders of magnitude) of gentamicin was measured on a sensor array consisting of four distinct PC sensors (L3, L13, L55 and L13 with nanoholes) following the test procedures in Section III.A to characterize the PC biosensors and the bio-molecular binding. The relative wavelength shift from the baseline for all four devices at different concentrations was extracted from the experimental spectra and plotted in Fig. 5. From this figure, it can be seen that for each type of PC biosensor, there is a linear response region where the resonance shift is almost linearly proportional to the log of the concentration, followed by a saturation region where there is only minimal shift with increased concentration. The data points were fitted with Langmuir equation [17], [27], [28]. From the fitting, the dissociation constant of the binding between gentamicin and anti-gentamicin was calculated. The results are in the range between 0.52 nM and 13 nM. The dose response curves show that L13 with nanoholes sensor produces significantly larger resonance shift and thus has higher sensitivity compared to other PC cavities without nanoholes; among PC cavities without nanoholes, longer cavities show larger

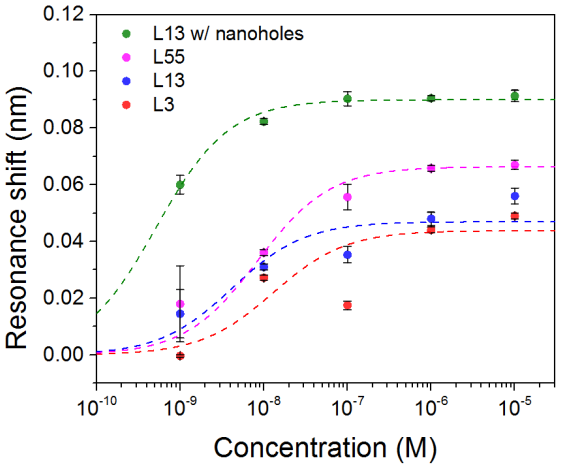

Fig. 5. Resonance shift of the four devices combined in the sensor array when target gentamicin antibiotics were bound to anti-gentamicin antibody on the sensor surface. The colored background and lines indicate the detection range for different PC microcavity biosensors.

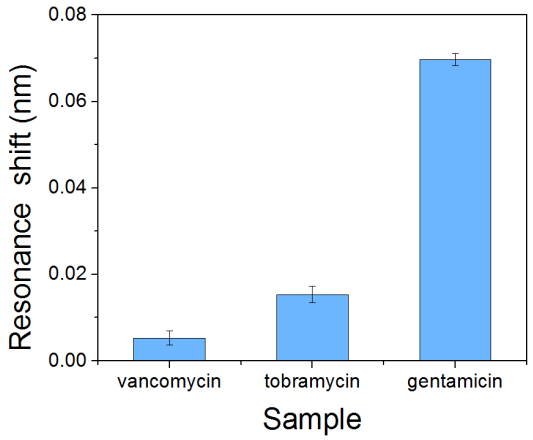

Fig. 6. Resonance shifts in L13 PC microcavity biosensors in the specificity test of gentamicin versus different target antibiotics.

shift and higher sensitivity than shorter cavities (from L55, L13 to L3). It can also be seen that L13 with nanoholes sensor begins to saturate at lower target concentration (∼10nM) than PC sensors without nanoholes (∼100nM to 1μM). It can be explained by the fact that the optical mode in L13 with nanoholes sensor concentrates in the small-size holes, which reduces the effective sensing region and makes it easier to saturate with biomolecules. With the sensor array containing PC sensors both with and without nanoholes, it is thus possible to cover a wider detection range than using a single type of PC sensor.

The specificity of the PC sensor was verified by measuring responses with antibiotics that do not bind to a specific antibody, as described in Section III.B. Fig. 6 shows the measurement result from the specificity verification test. Anti-gentamicin antibody probe is pre-immobilized on the sensor. The red-shift observed for tobramycin and vancomycin (non-specific bindings) are less than 0.02 nm, even though very high concentrations were introduced (1mM). The shift is much smaller than that from the specific binding of gentamicin (∼0.07 nm). Thus it shows good specificity in the detection method.

V. Conclusion

In summary, PC microcavities formed by 2D air holes on silicon-on-insulator substrates were demonstrated as biosensors to detect specific antibiotics in liquid form. The antibiotic, gentamicin, with molecular weight of only 478 g/mol, was successfully detected via its binding to its corresponding antibody pre-immobilized on the sensor surface. Detection range of concentrations was extended (0.1nM to 1μM) by combining different types of PC biosensors on a single silicon sensor chip with different minimum sensitivities. Specificity of the detection was also verified.

Our demonstrated silicon photonic biosensing platform can be implemented as a portable lab-on-chip sensing system. By integrating the PC biosensor chip with microfluidics, automated real-time testing can be realized. Furthermore, by utilizing PC filters [19], the device can function as a single-input single-output system, enabling high-throughput sensing tests with largely reduced optical peripheral components.

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