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Label-free aptamer sensor based on silicon microring resonators

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ABSTRACT

We present a label-free DNA aptamer sensor based on silicon microring resonators for the detection of human immunoglobulin E (IgE) and human thrombin. An array of silicon resonators consisting of three sensor microrings and one reference microring was fabricated and its surface sensitivity, detection limit and repeatability were investigated by the deposition of poly (sodium-4-styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) multilayers. The surface sensitivity and the limit of detection of the fabricated sensor ring are found to be 0.35 nm/ng mm^{-2} and 26 pg/mm^2 in air and 0.24 nm/ng mm^{-2} and 38 pg/mm^2 in water, respectively. We have demonstrated quantitative, sensitive and specific detection of human IgE and thrombin with aptamer modified silicon microring resonators over a broad range of antigen concentrations. The sensor array showed an experimental detection limit of 33 pM and 1.4 nM for IgE and thrombin, respectively. Multiplexing was also performed successfully with a mixed solution of IgE and thrombin and proved the sensor to be highly specific. We believe our silicon microring resonator technology in combination with aptamers offers a low cost solution for various point-of-care applications.

1. Introduction

Recently, silicon photonics has been applied to various biosensing applications by taking advantage of the highly sensitive evanescent field generated near silicon-on-insulator (SOI) photonic waveguides thanks to a contrast in refractive index between core silicon and its cladding, silicon dioxide or silicon nitride [1]. Silicon biophotonic devices such as silicon microring resonators [2,3], slot waveguides [4,5], Mach–Zehnder interferometer [6], and photonic crystal waveguides [7] provide a promising means for label-free detection of target molecules and for real-time monitoring of binding events that occur near the device surface. By utilizing the same foundries involved in manufacturing electronics, the field of silicon photonics is able to use these high volume and high quality processes to develop biophotonic sensors and open the market for very cheap and disposable in vitro diagnostic (IVD) devices [8].

Among silicon biophotonic devices, SOI microring resonators are extremely attractive as a biosensor due to their extremely small footprint and high sensitivity [3,9,10]. They can be integrated into a compact array, provide highly multiplexed detection within a single device and can be readily combined with fluidic components. Furthermore, the devices are fabricated by the standard CMOS technology, which ensures low-cost and scale-up capability of the sensor fabrication. The combination of low-cost fabrication and high sensitivity through small dimensions makes microring resonator a good candidate for disposable biosensor chips.

Silicon ring resonators are essentially refractive index-based (RI) sensors. Briefly, in a ring resonator, light propagates in the form of circulating waveguide modes, which result from total internal reflection of light along the curved boundary of the ring between the high (e.g. Si) and low refractive (e.g. SiO_2 or buffer) index media. This light is coupled into the cavity via an adjacent linear waveguide positioned within a couple of hundred nanometers. The circulating waves add constructively at those wavelengths that are divisors of the ring circumference. These are referred to as the ring resonant wavelengths. The circulating optical mode is sensitive to changes within the confines of the evanescent field. Therefore, biomolecule binding events (i.e. antibody-antigen interaction) on the surface of the microring lead to an increase in the effective RI and a shift in the resonant wavelength as illustrated in Fig. 1. The resonant wavelength shifts ($\Delta\lambda$) proportionally with the mass change of analytes bound to the surface. Or, in other words (as seen in Eq. (1)), with the effective refractive index of the resonator waveguide:

$$\Delta\lambda = \frac{\Delta n_{eff} \cdot \lambda_{res}}{n_g} \quad (1)$$

where Δn_{eff} is the change of the effective refractive index caused by analyte binding, λ_{res} is the initial resonance wavelength, and n_g is

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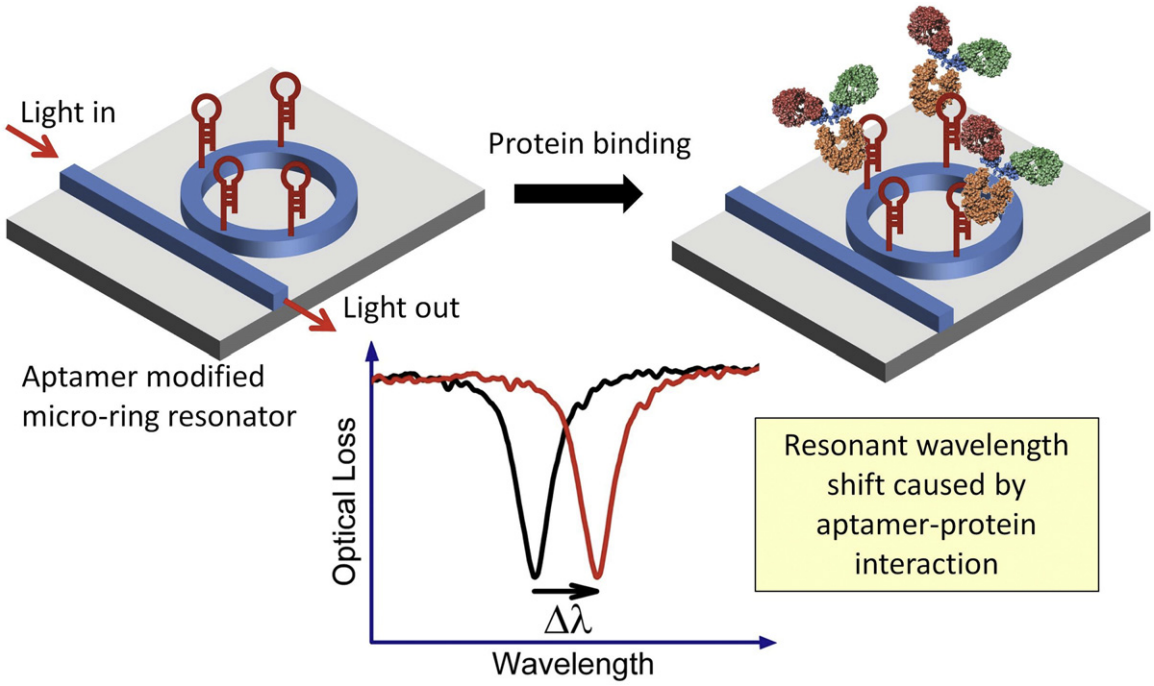

Fig. 1. Schematic diagram illustrating the principle of microring resonator biosensing and a corresponding optical loss spectrum.

the group index [2]. Therefore, by directly monitoring the resonant wavelength shift of a microring, it is possible to obtain quantitative information about the binding of molecules near the surface. SOI microring resonators typically exhibit a high Q factor ($>10^5$) owing to the geometry and the extremely low surface roughness. Their resonance peaks become extremely spectrally narrow which facilitate high resolution in terms of the spectral shift. This makes these devices very sensitive to the local refractive changes near the surface. The microring based biosensors have demonstrated highly sensitive and label-free detection of proteins [11–13], lectins [14] and nucleic acid sequences [15,16].

One of the main challenges in developing a biosensor device is the selection of ligands. Although antibodies are commonly used as recognition elements for biosensors due to their high specificity and affinity, they are expensive to produce, incompatible with some high-throughput approaches and often have limited shelf-life [17]. Therefore, the use of antibody as recognition ligands is one of the critical bottlenecks for the development of biosensors for clinical testing. Recently, aptamers (single-stranded RNA or DNA oligonucleotides), have been used as alternatives to antibodies for biosensors due to their several advantages over antibody based sensors [18,19]. Aptamers are very inexpensive to synthesize, relatively ease to isolate and modify, and resistant against denaturation and degradation. Their binding affinities and specificities can be easily manipulated and improved by rational design. Furthermore, immobilization of aptamers on the sensor surface is easier compared to antibodies because chemical modification of nucleic acid is simpler and more straightforward [20,21]. Thus, there is great promise to use these aptamers as ligands for the development of biosensors.

In this work, we present an aptamer-based silicon microring resonator sensor for the label-free detection of proteins. Sensor characteristics such as sensitivity, repeatability, and detection limit were investigated by depositing PSS/PAH multilayers. We chose well-described and widely studied IgE/anti-IgE DNA aptamer and human thrombin/anti-thrombin DNA aptamer as model systems to demonstrate the feasibility of aptamer sensor based on silicon micro-ring resonator [22,23]. An array of microring resonators was immobilized with anti-IgE aptamer or anti-thrombin aptamer or both. The resonant wavelength shift upon the addition of proteins was measured over 30 min and its peak position was plotted over a broad range of protein solutions. The apparent dissociation constant and maximum shift value were obtained by fitting the binding curves with Langmuir isotherm model. The detection limit of each pair was obtained experimentally. Furthermore, simultaneous detection of IgE and thrombin from mixed solution was demonstrated. The developed aptamer-based silicon microring resonators showed that IgE and thrombin proteins can be detected with high sensitivity and specificity, and our device can be used as a potential platform for point-of-care (POC) diagnostic applications.

2. Experimental

2.1. Materials

Immunoglobulin E (IgE) purified from human plasma (M.W. = 200 kDa) and alpha thrombin (human, M.W. = 37 kDa) were purchased from Abcam (Hong Kong) Ltd. 3-Aminopropyltriethoxysilane (APTES), glutaraldehyde (GAD) solution (50% wt in water), sodium cyanoborohydride solution (5.0 M in 1 M NaOH), ethanolamine, and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO). Poly (sodium-4-styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) were also purchased from Sigma–Aldrich. Other chemicals were analytical reagent grade and used as received. All samples and buffers were prepared using deionized water obtained from a Milli-Q water purification system. Anti-IgE DNA aptamer (5′-GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCC-$(CH_2)_7$-NH_2-3′) [24] and anti-thrombin DNA aptamer