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Ultracompact High-sensitivity Biosensing Based on Regenerable Laminated Bio-conjugation in Integrated Photonic Circuits

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Abstract

Biosensor based on micro-ring resonator platform has attracted much attention owing to its high sensitivity for identifying various toxins in the environment. Among various bio-toxins, Botulinum neurotoxin (BoNT) are one type of poisonous substances causing the life-threatening neuromuscular disease and probably used as biological weapon. In our study, a silicon based micro-ring resonator is designed, fabricated and demonstrated to detect BoNT in water sources. First, the capture antibody is labelled on the surface of silicon waveguide for capturing BoNT. The amount of BoNT bound on the surface results in the change of the effective refractive index. As a result, the concentration of toxins can be sensed based on the resonant shift response. Before involving the detection antibody in the assay, the BoNT concentration can be detected in the range from 200 ng/mL to 1 µg/mL. For further improving LOD, a signal enhancement strategy is to use biotin conjugated detection antibody to form a sandwich structure, and then to bind streptavidin-HRP and biotin conjugated anti-HRP antibody. LOD can be improved to 30 ng/mL after linking the detection antibody. After associating with the layer-by-layer structure composed by HRP and anti-HRP antibody on the sandwich assay, LOD can be improved to several pg/mL. Based on this strategy, the process of signal enhancement is flexible to achieve target LOD. And it also can be regenerated and reused after a cycle of detection. Therefore, this platform shows a high potential to apply for robust, real-time and high sensitive detection for various bio-toxins in water sources.

Keyword: Biosensor, micro ring resonator, botulinum neurotoxin, enhancement strategy, laminated bio-conjugation, regenerable

Introduction

Botulinum neurotoxins (BoNTs) are one of the exotoxins produced by the bacterium *Clostridium botulinum*.^[1] It has been classified as one of the six highest-risk threat agents of bioterrorism by the Centers for Disease Control and Prevention of the United States, with a median lethal dose (LD50) of approximately 1 ng per kg of body weight and are the cause of the life-threatening neuromuscular disease botulism.

Faced with the threat of potential biological weapon, increased attention has been given to multiplexed biomolecular analysis because of its potential to revolutionize areas of human health, such as disease diagnosis and therapeutic selection. Whispering gallery mode sensors are a class of powerful analytical techniques defined by the measurement of changes in the local refractive index at or near the sensor surface.^[2]

When functionalized with target-specific capture agents, analyte binding can be measured with very low limits of detection. In our study, a silicon based micro-ring resonator is designed, fabricated and demonstrated to detect BoNT in water sources.

Detection Principle and System Design

A mouse-derived antibody F1-40 (IgG1 monoclonal) was selected as the capture antibody. The surface treatment steps are shown in **Figure 1**. The chip was treated with oxygen plasma for 5min and then dipped into MPTMS (1% v/v) in toluene for 4h. After washing the chip with ethanol in sonication bath, GMBS

solution, the bifunctional cross linker, was dropped and left on chip surface for 1h. After another washing step same as before, the chip active surface was incubated with 50 μ g/ml IgG1 at 4 $^{\circ}$ C overnight. Finally, the chip was put into 1mg/mL BSA in PBS solutions as blocking buffer at least 1h until used.^[3]

Figure 1 Surface treatment steps

In the experiments, pre-treated chip was washed by PBS, and then put into homemade holder which consists of stainless steel substrate and PTFE gasket. The holder was attached onto the temperature control model. The total set-up was adhered onto the optical alignment stage. For optical alignment system, two taper fibres were aligned to both ends of bus waveguide. They were adjusted to the particular positions which results in maximum intensity in the output spectrum. The running buffer is injected by the syringe pump to remove the unwanted contamination or unspecific binding from the chip.

Figure 2 System Design

Fabrication Process

Figure 3 Fabrication process

The fabrication process flow of the nano-silicon-photonic waveguide circuit on 8 inch SOI wafers is summarized in **Figure 3**. The surface layer of crystalline silicon has a thickness of 220nm, the buried SiO_2 has a thickness of $2\mu\text{m}$ and the substrate silicon has a thickness of $700\mu\text{m}$. Grating coupler is first patterned via deep UV photolithography. Followed by reactive ion etching (RIE) on silicon structure layer. After photo resist strip and clean process. The pattern of grating coupler is transferred on the silicon layer. 70nm of silicon dioxide is deposit on top via low temperature plasma-enhanced chemical vapour deposition (PECVD), which used as hard mask for silicon layer etching. Then silicon waveguide is patterned by lithography. Two step RIE etching transfer the pattern on SiO_2 hard mask first and then onto the silicon layer. Hard mask is used to ensure the accuracy of RIE etching. Rib structure is patterned after waveguide structure, similar to the previous process. After this step, the silicon structure layer is finished. $2\mu\text{m}$ thickness silicon dioxide is deposited on top of the structure by high density plasma CVD. A trench at edge of chip is etched and 40nm thickness of Al_2O_3 is deposited by atomic layer deposition (ALD). This process is to protect edge of chip during dicing process. Then window structure is patterned on Al_2O_3 layer, which is used as hard mask for following vapour HF release process. The last step is to expose the waveguide structure. Vapor HF is used to etch through the silicon dioxide layer.^[4]

Results and Discussion

First, the capture antibody is labelled on the surface of silicon waveguide for capturing BoNT. The amount of BoNT bound on the surface results in the change of the effective refractive index. As a result, the concentration of toxins can be sensed based on the resonant shift response. Before involving the detection antibody in the assay, the BoNT concentration can be detected in the range from 200 ng/mL to 1 $\mu\text{g/mL}$ as shown in **Figure 4**.

Figure 4 Peak shift versus time and concentration

For further improving LOD, a signal enhancement strategy is to use biotin conjugated detection antibody to form a sandwich structure, and then to bind streptavidin-HRP and biotin conjugated anti-HRP antibody. LOD can be improved to 30 ng/mL after linking the detection antibody and streptavidin-HRP molecule as shown in **Figure 5**.

Figure 5 Signal enhancement peak shift versus time and concentration

After associating with the layer-by-layer structure composed by HRP and anti-HRP antibody on the sandwich assay, LOD can be improved to several pg/mL. **Figure 6** shows the ideal structure and some test result of it. Based on this strategy, the process of signal enhancement is flexible to achieve target LOD. And it also can be regenerated and reused after a cycle of detection. Therefore, this platform shows a high potential to apply for robust, real-time and high sensitive detection for various bio-toxins in water sources.

Figure 6 Layer-by-layer structure and part test result

Conclusions

In our study, we propose to use a layer-by-layer structure composed of biotin conjugated detection antibody, streptavidin-HRP and biotin conjugated anti-HRP antibody combination as a new strategy for signal enhancement on a micro-ring resonator biosensor. By using this strategy, the process of signal enhancement is flexible to achieve target LOD. And it also can be regenerated and reused after a cycle of detection. Therefore, this platform shows a high potential to apply for robust, real-time and high sensitive detection for various bio-toxins in water sources.

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