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New method for online regeneration of silicon-based nanophotonic biosensors †

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Abstract: The optimal development of biosensors is a costly and time-consuming task, since an enormous amount of experiments is required. Therefore, the possibility of reusing the biosensors is highly desirable. In this work, a protocol based on the use of formamide for the regeneration of nanophotonic biosensors used for oligonucleotides detection is presented. This protocol was carried out online using the microfluidic system used to drive the target samples to the nanophotonic biosensor, thus allowing the possibility of running several experiments in a row using the same biosensor.

Keywords: nanophotonic biosensor; regeneration; oligonucleotides; formamide; dehybridization; silicon-photonics

1. Introduction

In the last years, it has been a growing interest in the development of biosensors for different applications in medicine, environmental sensing or food testing, among others [1]. Bridging the gap between the laboratory development of the biosensors to their real application is expensive and time-consuming. Besides their design, manufacture and biofunctionalization, an extensive experimental testing work is required. One way to reduce the amount of resources and time required for that testing is reusing the biosensors. With this aim, different regeneration strategies have been explored by several groups [2-4]. However, most regeneration protocols require removing the transducers from the experimental system and to reassemble them again in the system after the regeneration is performed.

In this work, we pursue a strategy to reuse silicon-based nanophotonic biosensors functionalized with molecular beacon (MB) probes for the detection of oligonucleotides targets, without removing them from the experimental measuring system. More specifically, in this work we will focus on the regeneration of nanophotonic sensors to be used in the detection of microRNA (miRNA) targets. This strategy aims at performing a so-called online regeneration, which not only allows saving time, but also reducing the sensor-to-sensor variance in the experimental sensing results, what is especially useful when testing similar levels of analyte. Chemical regeneration based on formamide (FA) was the strategy explored in this study. FA is a denaturing agent for nucleic acids, which is commonly used in DNA solutions [5]. However, little is known about FA as denaturing agent for miRNA bound to MB probes immobilized on silicon surfaces as in the case we are interested in [6]. Our study consisted of, after running a typical miRNA sensing experiment, flowing FA in water to dehybridize the probes and regenerate the sensor for performing further experiments.

2. The biosensor

The principle of operation of the label-free optical biosensor used in this work is based on the interaction of the evanescent wave of a guided mode with the refractive index (RI) changes produced by the sample to be detected in the surroundings of the sensing surface of the nanophotonic structure. In particular, the transducer of this biosensor (sensing structure) is a silicon photonic bandgap (PBG) nanostructure manufactured in a silicon on-insulator (SOI) wafer, as shown in Figure 1(a). This nanostructure was created by periodically introducing a modulation in the refractive index of a 1D photonic structure. It consisted of a single mode waveguide placed on top of a silicon oxide lower cladding with straight transversal elements [6, 7], as shown in Figure 1(b). The photonic detection is based on measuring the shift of the upper edge of the PBG. The bioreceptors employed were MB probes, immobilized by thiol-ene coupling (TEC) chemistry to the previously derivatized surfaces of the PBG sensing structures [8].

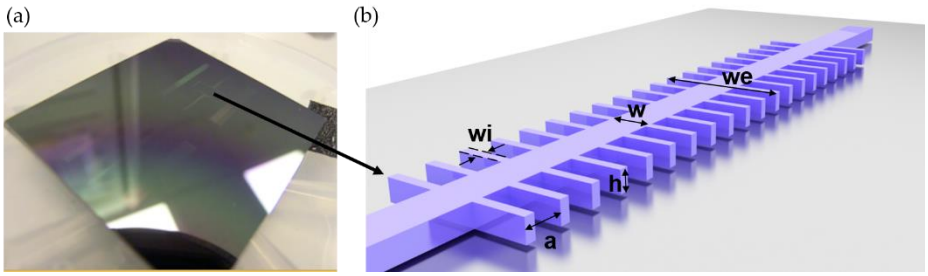

Figure 1. (a) Photonic chip, manufactured in a SOI wafer, containing the nanophotonic sensing structures. The black arrow indicates the position where one of the photonic sensing structures is located. (b) Schematic representation of the 1D PBG sensing structure with descriptive key dimensions.

3. Experimental setup

For testing the biosensors, a polydimethylsiloxane (PDMS) flow cell having a microfluidic channel was attached to the silicon chip to drive the sample to the sensors. The pumping system used was a commercial syringe pump. The optical characterization setup consisted of a continuous sweep tunable laser with the corresponding optical fiber and the optics to transmit and focus the laser light into the access grating couplers. For the detection, an objective was used to collect and focus the transmitted light from the output grating couplers into an infrared (IR) camera. The convenient software to control this setup for optical interrogation is programmed in LabVIEW allowing a continuous acquisition of the spectral response of the sensors.

4. Test and results

The test consisted of firstly performing an experiment for the photonic detection of the miRNA target (miR-155) that consisted of two steps: first, saline-sodium citrate (SSC) buffer 5x was flowed to obtain the baseline of the sensing structures; second, a solution of 0.5μM of miRNA target in SSC 5x was flowed at a flow rate of 20ul/min over the chip for 15 minutes. Figure 2(a) shows the evolution of the PBG edge position during this experiment, where we can see that the PBG is shifted once the miRNA target reaches the sensors and that a typical hybridization curve is obtained. Subsequently, the online regeneration test was performed by flowing FA 25% in UPW (ultrapure water) for 20 minutes (UPW was flowed before and after the FA regeneration for properly washing the surface). The photonic response showed by the sensors during this regeneration protocol was monitored in real-time (see Figure 2(b)), showing a negative PBG shift during the FA flow that can be ascribed to the dehybridization of the miRNA targets from the MB probes. Finally, the experiment for the detection of miRNA targets was repeated, obtaining the results shown in Figure 2(c). Hybridization of the target miRNAs can be observed again, but a signal loss of 50% is obtained after regenerating the sensor.

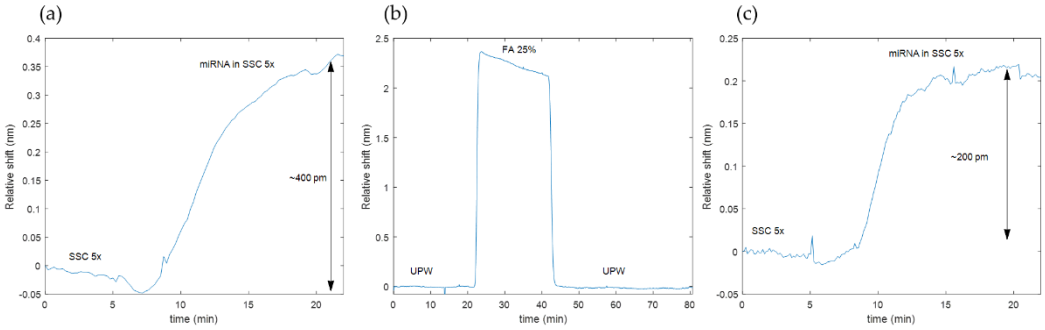

Figure 2. Time evolution of the PBG edge position of the sensing structure: (a) Acquired during the initial experiment for detection of miRNA; (b) Acquired during the online regeneration with FA in UPW; (c) Acquired during the second experiment for detection of miRNA (after regeneration).

5. Discussion

These results confirm the feasibility of the online regeneration based on FA, allowing the completion of several experiments in a row. Nevertheless, further optimization work is required to reach a better performance of the protocol. Taking these results as a starting point, different concentrations of FA and different duration of the regeneration period should be explored and also the combination of this chemical regeneration with thermal changes that favours the denaturalization of the hybridized oligonucleotides [5].

6. Conclusions

We present a study of a convenient regeneration protocol for nanophotonic biosensors for oligonucleotides detection, which can be easily integrated as an additional step in the experimental microfluidic system required for the proper testing of this type of biosensors. This development allows saving work of highly skilled personnel and reducing costs of materials and reagents for the preparation of the biosensors, which is an important advance in the time-consuming and expensive processes of development and optimization of these biosensors.

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