Live Tracking Biofunctionalization and Label-Free Protein Detection Performed by a Nanophotonic Biosensor †

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Abstract: A label-free biosensor based on silicon-on-insulator (SOI) photonic bandgap (PBG) structures is performed for specific protein detection. First, the SOI sensing surface is functionalized using triethoxyvinylsilane (TEVS) organosilane. Then, a UV light photocatalysed immobilization of polyclonal half anti-bovine serum albumin (haBSA) antibodies is performed. Finally, a direct detection of target BSA antigen is carried out. Both the immobilization and the detection steps are monitored by making a continuous tracking of the PBG edge shift. In order to confirm the recognition of the antigen by the immobilized antibody, a fluorophore-labelled secondary antibody is flowed at the end of the experiment in order to perform a confirmation fluorescence test after the photonic detection.

Keywords: photonic sensor; photonic bandgap; silicon on insulator; biofunctionalization; photocatalysis; half antibodies

1. Introduction

During the last years, significant progresses have been made in the development of photonic-based biosensing devices, since they offer several advantages over the conventional methodologies such as PCR or ELISA, which are expensive, bulky, time consuming, and lab centralized. Evanescent wave based photonic biosensors can provide a high sensitivity detection in a label-free scheme and with a high multiplexing potential for parallelized analysis, making them suitable for the development of high performance Lab-on-a-chip devices [1]. Additionally, they are typically based on standard silicon technology fabrication, allowing affordable mass production and miniaturization [2].

However, in order to achieve a high sensitivity, as well as a high selectivity and accuracy, in a biosensing platform, a trustworthy functionalization protocol for the immobilization of the receptors on the sensing structure must be available. That would allow analyzing chemical and biological samples without any pretreatment (as for example labelling or filtering). Among the different procedures for the biofunctionalization of sensing structures, covalent strategies for the immobilization of bioreceptors provide several advantages in terms of non-specific interactions restriction and thickness reduction of the recognition layer compared to other simpler strategies based on physical adsorption.

Here, we report our work towards the development of high sensitivity and selective photonic-based biosensors. We have used photonic bandgap (PBG) structures as sensing elements, since they have emerged as one of the most sensitive photonic configurations for a label-free detection [3]. In these structures, the introduction of a periodic modulation in the refractive index of the photonic structure gives rise to the appearance of a rejected spectral band, the so-called PBG [4], whose position will depend on the refractive index of the surrounding medium with a high sensitivity. In order to provide selectivity to the PBG sensing structures, antibodies specific to Bovine Serum Albumin (BSA) have been immobilized on the sensors surface. However, instead of using whole antibodies, half BSA antibodies (haBSA) obtained using tris(2-carboxyethyl)phosphine (TCEP) reduction have been used in order to obtain a higher surface coverage density, a lower thickness of the recognition layer and to have a proper orientation of the antibodies binding sites. The linkage of the haBSA antibodies to the sensors has been performed via the thiol moieties available after the TCEP reduction by covering the surface with vinyl groups using triethoxyvinyl-silane (TEVS) [5]. Upon the illumination of the sensor with UV light, the reaction between the thiol and the vinyl groups takes place and the covalent immobilization of the haBSA antibodies is produced leading to a thioeter for the covalent immobilization. Finally, the sample containing the target BSA antigens is flowed over the PBG sensing structure in order to be specifically recognized by the haBSA antibodies immobilized on the sensor surface. Both the haBSA antibodies immobilization and the BSA recognition steps have been monitored in continuous using the PBG sensor, what has allowed us to experimentally demonstrate the binding of the haBSA antibodies to the surface only upon the excitation with UV light and the subsequent recognition of the BSA targets by the biofunctionalized photonic biosensor.

2. Materials and methods

Figure 1 shows pictures of the fabricated photonic chip containing the PBG sensing structures. It has been created in a silicon-on-insulator (SOI) substrate using e-beam lithography with an acceleration voltage of 100 keV and inductively coupled plasma etching of the top silicon layer. The structural parameters of the PBG sensing structures are height h = 220 nm, waveguide width w = 450 nm, period a = 380 nm, transversal elements length w_e = 1500 nm and transversal elements widths ranging from wi = 80 nm to w_i = 140 nm. These structural parameters provide PBG edges located in the wavelength range between 1500 nm and 1600 nm, where our experimental characterization equipment operates. The chip is accessed at the input and the output via 70 nm-deep shallow etch 1D grating couplers [6].
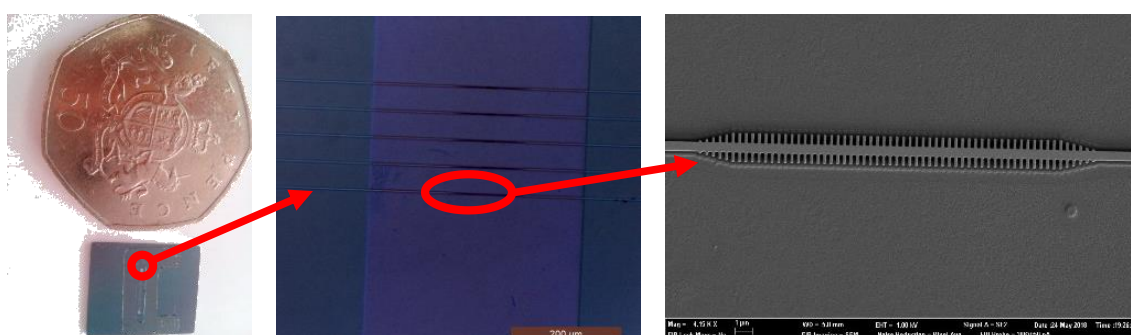

Figure 1. (Left) Image of the photonic chip fabricated in SOI technology. The circle depicts the position of each PBG sensors groups. (Center) Microscope image of a PBG sensors group. The circle depicts an individual PBG sensing structure. (Right) Scanning Electron Microscope (SEM) image of a fabricated PBG sensing structure.

To obtain a vinyl-terminated monolayer on the surface of the SOI PBG sensing structures, where the haBSA antibodies will be immobilized via their thiol moieties using a light-assisted thiolene coupling (TEC) reaction, the photonic chip is silanized using triethoxyvinyl-silane (TEVS). The steps of the silanization process used in this work are depicted in Figure 2. Typically, silanization processes

are carried out using an organic anhydrous solvent as carrier for the organosilane, as for example toluene [7]. However, the use of organic solvents can lead to several problems such as vertical polymerization, what produces a thicker silane layer that will increase the distance between the photonic sensors and the target analytes that will reduce the sensitivity, and the generation of organic waste [8-10]. Taking these problems into account, we have performed the silanization using water as carrier for the TEVS organosilane. Despite the solubility of TEVS in water is not complete, the exchange of its triethoxy groups with OH groups from water makes it stable. In order to have an optimal triethoxy ↔ OH exchange ratio and to finally obtain a compact vinyl monolayer on the surface, the pH of the solution is adjusted to 8 using KOH or NaOH. Therefore, the final silanization process consisted on immersing the SOI chip in 1% TEVS in MilliQ water (pH adjusted to 8 by adding 100 μL from 1M KOH mother dissolution) during 1 hour and finally curing it at 110 °C during 1 hour for condensation and water excess evaporation. Note that, before performing the silanization process, the SOI chip was cleaned in a piranha solution (H_2SO_4/H_2O_2:1/3) for 20 minutes and then activated using O_2 plasma for 10 minutes.
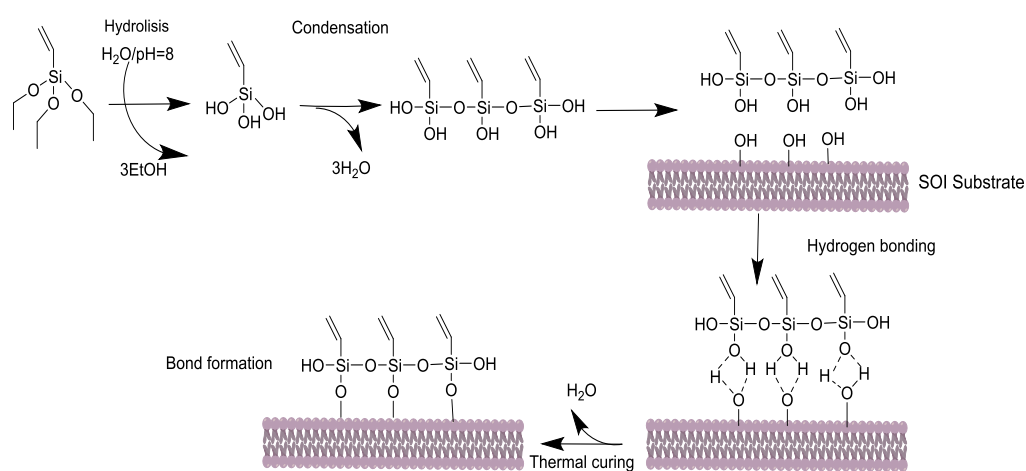

Figure 2. Steps of the silanization process used to create a vinyl-terminated monolayer on the surface of the SOI sensing structures.

In order to covalently immobilize the BSA antibodies over the sensor surface using the TEC reaction, they are first divided using the tris(2-carboxyethyl)phosphine (TCEP) reduction method described in [5]. After the TCEP reduction, thiol moieties will be available on the resulting haBSA antibodies for their immobilization over the vinyl-terminated surface by mean of UV light (254 nm) photocatalysis. Figure 3 schematically shows the haBSA immobilization scheme employed.
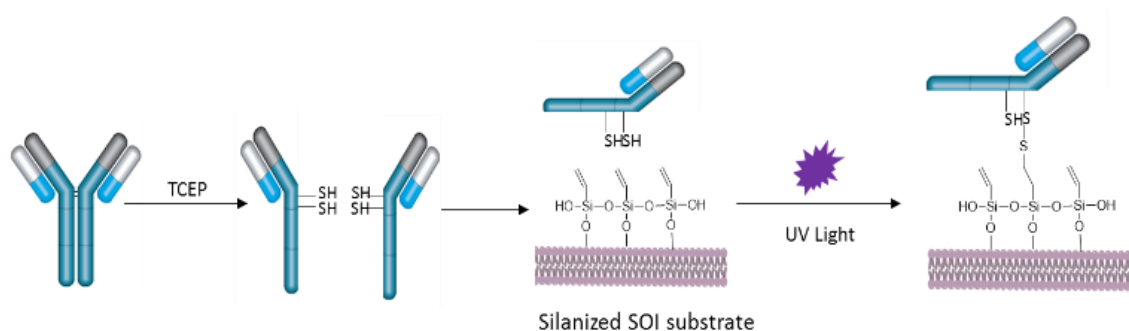

Figure 3. Schematic representation of the process used for the covalent immobilization of the haBSA antibodies on the PBG sensors surface.

Finally, once the haBSA bioreceptors are immobilized on the surface, the target BSA antigen is flowed over the chip in order to be detected by the PBG sensing structures.

3. Results and discussion

Figure 4 shows images of the characterization of the chip carried out after performing the TEVS silanization process described above. The water contact angle (WCA) test indicates an increase of the hidrophobicity of the surface, what confirms the coverage with vinyl groups. On the other hand, the Atomic Force Microscope (AFM) characterization of the surface shows a very low roughness, also confirming the proper creation of the organosilane layer. Additionally, ellipsometry measurements were carried out, providing a TEVS layer thickness of 0.33±0.03 nm, confirming again the creation of a compact organosilane monolayer.
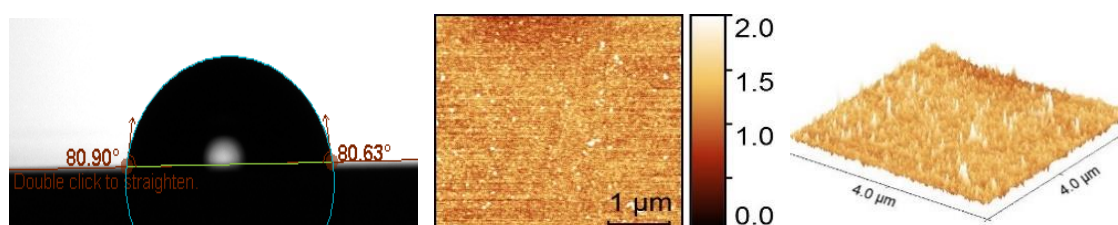

Figure 4. (Left) WCA test confirming the hydrophobicity increase of the surface. (Right) AFM topography characterization of the silanized SOI surface.

The silanized chip is then assembled with a PDMS-based microfluidic cell used to flow the reagents over the photonic chip while its sensing response is continuously monitored. The optical characterization set up used for the interrogation of the photonic sensing chip mainly consists on a continuous sweep tunable laser and an infrared camera synchronized with it in order to acquire the spectral response of the different photonic structures within the chip and to track their spectral shift during the sensing experiments.

Figure 5 shows a representative spectral evolution of the PBG edge for one of the sensing structures within the chip during the haBSA immobilization step. Initially, Phosphate Buffered Saline (PBS) 1x is flowed over the chip in order to obtain the initial baseline. Then, the solution containing the reduced haBSA (200 μg/mL in PBS 1x) is flowed. As it can be observed in Figure 5, no photonic sensing response is obtained at that moment, despite both the surface vinyl groups and the thiol moeities of the haBSA antibodies are present. It is not until the photonic chip is irradiated with UV light that the vinyl-thiol reaction is photocatalyzed and the haBSA antibodies are immobilized on the sensor surface, what is translated into a shift of the PBG position. Finally, PBS 1x buffer is flowed again in order determine the net spectral shift and to remove any excess of haBSA antibodies.
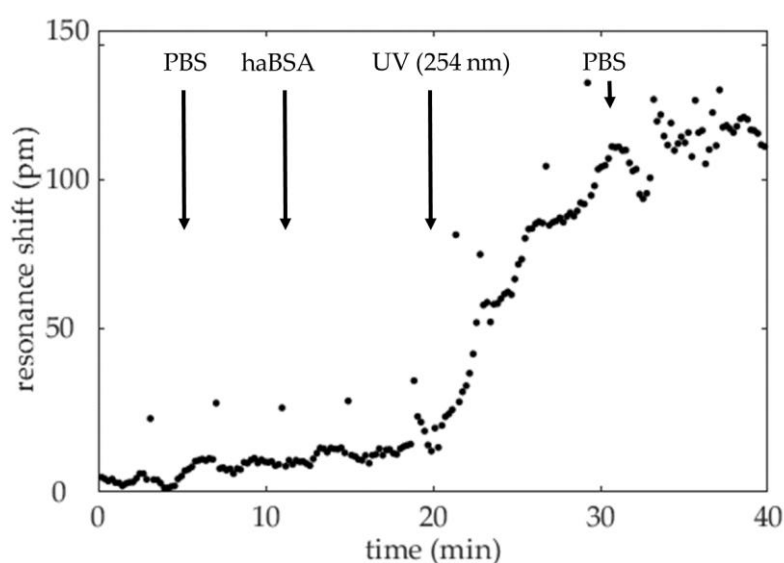

Figure 5. Temporal evolution of the PBG spectral shift during the light-assisted haBSA immobilization process. haBSA are injected at minute 10 and UV light source is switched on at minute 20.

Finally, BSA 1 μg/mL in PBS-T (PBS + 0.01% Tween 20) is flowed over the biofunctionalized sensing surface in order to characterize its specific recognition by the immobilized haBSA antibodies (the buffer is changed from PBS to PBS-T to prevent non-specific interactions and BSA adsorption). Figure 6(a) depicts representative results for this experiment, where we can see that the target BSA is properly detected by the PBG sensing structure. In order to corroborate the haBSA-BSA recognition, a fluorophore-labelled secondary BSA antibody is then flowed over the photonic chip for fluorescence characterization (see Figure 6(b)).
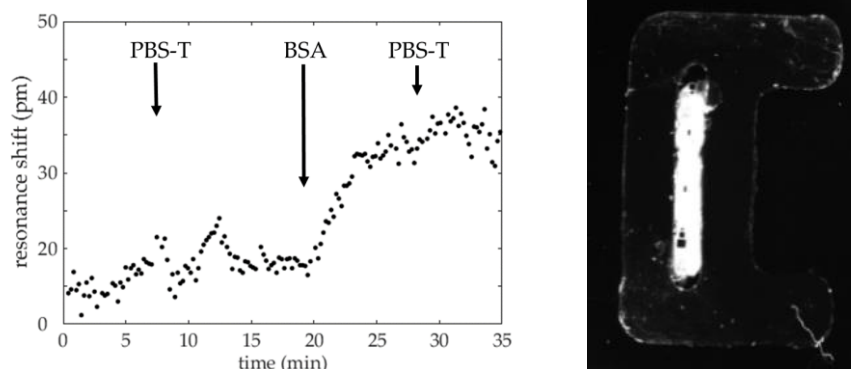

Figure 6. (Left) Temporal evolution of the PBG spectral shift during the BSA detection experiment. (Right) Fluorescence test carried out after binding a fluorophore-labelled secondary aBSA antibody to the recognized BSA target.

4. Conclusions

In this work, the development of a PBG photonic biosensor for the label-free detection of proteins is presented. This is, to our knowledge, the first time that a photonic sensing structure biofunctionalized with half antibodies as recognition layer is reported. The use of half antibodies provides several very important benefits for the development of higher sensitivity biosensors, as a higher surface coverage density, a lower thickness of the recognition layer and a proper orientation of the antibodies binding sites. Additionally, the light-assisted immobilization of the haBSA antibodies on the sensing surface using TEC biofunctionalization has been tracked in-live, confirming that the reaction between the vinyl groups on the surface and the haBSA thiol moieties are only produced upon UV light photocatalysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jokerst, J.V.; Jacobson, J.W.; Bhagwandin, B.D.; Floriano, P.N.; Christodoulides, N.; McDevitt, J. T. Programmable nano-bio-chip sensors: analytical meets clinical. *Anal. Chem* **2010**, 82 (5), 1571, doi: 10.1021/ac901743u.
2. Dutta, H.S.; Pal, S. Design of a highly sensitive photonic crystal waveguide platform for refractive index based biosensing, *Opt. Quantum Electron* **2013**, 45(9), 907–917, doi: 10.1007/s11082-013-9697-x.
3. Wang, X.; Flueckiger, J.; Schmidt, S.; Grist, S.; Fard, S.T.; Kirk, J.; Doerfler, M.; Cheung, KC.; Ratner, DM.; Chrostowski, L. A silicon photonic biosensor using phase-shifted Bragg gratings in slot waveguide. *J. Biophotonics* **2013**, 6 (10), 821, 8, doi: 10.1002/jbio.201300012.
4. Povinelli, M.; Johnson, S.; Joannopoulos, J. Slow-light, band-edge waveguides for tunable time delays Opt. Express **2005**, 13(18), 7145–7159, doi: 10.1364/OPEX.13.007145.
5. Alonso, R.; Jiménez-Meneses, P.; García-Rupérez, J.; Bañuls, M.J.; Maquieira, Á. Thiol-ene click chemistry towards easy microarraying of half-antibodies, *Chem.Comm* **2018**, 54, 6144-6147, doi: 10.1039/c8cc01369a.
6. García, J.; Sanchis, P; Martínez, A.; Martí, J. 1D periodic structures for slow-wave induced non-linearity enhancement Opt. Express **2008,** 16(5), 3146–3160, doi: 10.1364/OE.16.003146.

7. Haensch, C.; Hoeppener, S.; Schubert, U.S. chemical modification of self-assembled silane based monolayers by surface reactions. *Chem Soc Rev* **2010**, 39, 2323-2334, doi: 10.1039/B920491A.

8. Kim, J.; Seidler, P.; Wan, L.S.; Fill, C. Formation, structure, and reactivity of amino-terminated organic films on silicon substrates. *J Colloid Interface Sci* **2009**, 329, 114, doi: 10.1016/j.jcis.2008.09.031.

9. Phaner-Goutorbe, M.; Dugas, V.; Chevolot, Y.; Souteyrand, E. Silanization of silica and glass slides for DNA microarrays by impregnation and gas phase protocols: A comparative study. *Mater Sci Eng* C **2011**, 31, 384-390, doi: 10.1016/j.msec.2010.10.016.

10. Wang, A.; Tang, H.; Cao, T.; Salley, S.O.; Ng, K.Y.; In vitro stability study of organosilane self-assemble monolayers and multilayers, *Journ Coll Interf Sci* **2005**, 291, 438, doi: 10.1016/j.jcis.2005.05.008.