1D Periodic Corrugated Waveguides for Real-Time Detection of Increasing Concentrations of Thrombin

Paula Martínez-Pérez, Maribel Gómez-Gómez, Luis Torrijos-Morán, Todora Angelova, Amadeu Griol, Juan Hurtado, Laurent Bellieres and Jaime García-Rupérez

Nanophotonics Technology Center, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
Author e-mail address: paumarp3@ntc.upv.es

Abstract: An aptasensor based on periodic 1D corrugated waveguides is demonstrated to be suitable for the specific and continuous detection of thrombin at nM concentrations in real time with an estimated detection limit of 33.5 pM.

1. Introduction

Thrombin is the central enzyme of coagulation cascade and disruptions of its formation has been related to the onset of different pathologies [1,2]. Due to this fact, the development of a reliable system for the characterization of thrombin generation process has been pursued. Indeed, commercial methods already exists, but they are not sensitive enough to monitor the initial stage of thrombin formation, known as the initiation phase, when the concentration is in the nanomolar range (from 1 nM to 20 – 30 nM) [3]. In this work, a photonic aptasensor is proposed to overcome such limitation. It consists of a 1D photonic crystal (PC) corrugated waveguide with thrombin binding aptamer (TBA) as bioreceptor on its surface. Using this system, we demonstrated that continuously-increasing thrombin concentrations ranging from 270 pM to 27 nM can be detected in real time with a short response time (~2 min) and an estimated limit of detection (LOD) of 33.5 pM.

2. Methodology

Arrays of 16 1D PC corrugated waveguides are created on a silicon-on-insulator (SOI) wafer by e-beam lithography and inductively coupled plasma etching of the top silicon layer. Such corrugated waveguides consist of a single-mode waveguide (220-nm height and 460-nm wide) with periodic transversal elements (380-nm periodicity, 1500-nm length and four possible widths: 140 nm, 120 nm, 100 nm or 80 nm) and are accessed at the input and the output via 70-nm-deep shallow etched 1D grating couplers.

Regarding the biofunctionalization of their surface, a silanization step is carried out employing 3-aminopropyltriethoxysilane (APTES) after the surface activation by immersing the sample in piranha solution (H_2SO_4:H_2O_2, 3:1). Then, glutaraldehyde (GA) is employed as crosslinker in order to attach the 3'-amine-modified TBA probes with the sequence 3'-$(T)_{12}$-GGTTGGTGTGGTTGG-5' [4].

To carry out optical measurements, a software implemented in LabVIEW is employed to control a wavelength-tunable laser, as light source, and an infrared camera coupled to a 20x objective, to collect the transmitted light by the PC structures [5].

Thrombin solutions to be detected are flowed using a syringe pump working in withdraw mode at 20 µl/min through a flow cell, consisting of a PMMA piece with and inlet and an outlet tube, and an adhesive tape that keeps tightly bound such PMMA piece to the photonic chip and creates a channel over the surface of PC structures.

3. Results

In order to carry out the biorecognition measurements, the PBG edges of 4 PC structures are employed because they show a well-defined shape, which eases the monitorization of their position. Such PBG edges are named henceforth as PC1, PC2, PC3 and PC4.

Firstly, a solution of 1 mg/ml bovine serum albumin (BSA) in 1x PBS, hereafter referred as blocking solution (BS), is flowed over the PC structures to block the remaining reactive groups of GA molecules and hence, avoid the unspecific attachment of thrombin to the surface. Then, a baseline is stablished with such BS and four different solutions of thrombin in BS at a concentration of 27 pM, 270 pM, 2.7 nM and 27 nM are flowed consecutively in order to emulate the continuous thrombin production that occurs during the initiation phase of the thrombin generation process. When 27 pM solution is flowed, no change in the PBG edge position is observed. By contrast, an average shift of the PBG edges of 12 pm, 23 pm and 48 pm is observed when 270 pM, 2.7 nM and 27 M thrombin solutions are respectively flowed (see Fig.1). This demonstrates that the aptasensor is capable of recognizing thrombin when its concentration ranges from 270 pM to 27 nM in real time. An exponential relation can be observed between the shift experience by the PBG edges and the concentration of thrombin (R^2=0.99), with a linear relation (R^2=0.99) when thrombin concentration is below 2.7 nM. In such linear range, a sensitivity of 12.6

pm/nM is calculated. The LOD was estimated to be 33.5 pM and the response time, as the time that the aptasensor requires to experienced half of the maximum shift for each concentration, is ~2 min.

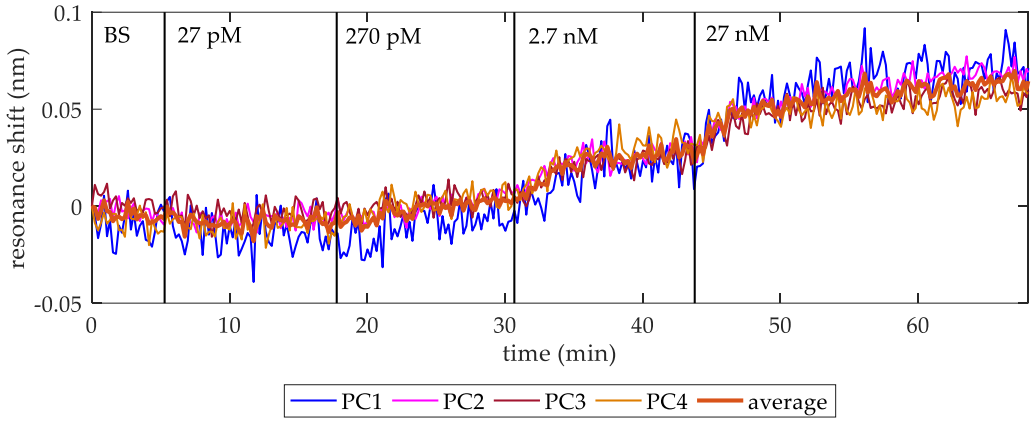

Fig. 1. Resonance shift experienced by PBG edges of four PC structures when four different concentrations of thrombin are consecutively flowed.

In order to check that such biorecognition is specific, the experiment was repeated employing a photonic chip in which only PC1 and PC2 have TBA on their surface, while PC3 and PC4 only have APTES and GA and lack TBA. In this occasion, only the highest thrombin concentration is flowed after flowing BS and only those PC structures with TBA on their surface experienced a shift of their PBG edges, demonstrating the specificity of the biorecognition.

4. Conclusions

The results reported here demonstrate the suitability of this photonic aptasensor based on 1D PC corrugated waveguides for the continuous and specific detection of thrombin in real time. Concentrations ranging from 270 pm to 27 nM were detected with a response time of ~2 min and an estimated LOD of 33.5 pM. The nanomolar range in which this aptasensor works and its working parameters make it a good candidate to monitor thrombin during the initiation phase of thrombin generation process.

Funding

This research was supported by a co-financed action by the European Union through the operational program of the European Regional Development Fund (FEDER) of the Valencian Community 2014-2020, the Generalitat Valenciana through the PROMETEO project AVANTI/2019/123 and the grant ACIF/2019/009, and by Universitat Politècnica de València through grants PAID-01-17 and the project OCUSENSOR.

5. References

[1] S. Danckwardt, M. W. Hentze, and A. E. Kulozik, "Pathologies at the nexus of blood coagulation and inflammation: Thrombin in hemostasis, cancer, and beyond," J. Mol. Med. **91**, 1257–1271 (2013).

[2] K. Göbel, S. Eichler, H. Wiendl, T. Chavakis, C. Kleinschnitz, and S. G. Meuth, "The coagulation factors fibrinogen, thrombin, and factor XII in inflammatory disorders-a systematic review," Front. Immunol. **9**, (2018).

[3] J. Kintigh, P. Monagle, and V. Ignjatovic, "A review of commercially available thrombin generation assays," Res. Pract. Thromb. Haemost. **2**, 42–48 (2018).

[4] A. T. Ponce and K. L. Hong, "A mini-review: Clinical development and potential of aptamers for thrombotic events treatment and monitoring," Biomedicines **7**, (2019).

[5] Á. Ruiz-Tórtola, F. Prats-Quílez, D. González-Lucas, M. J. Bañuls, Á. Maquieira, G. Wheeler, T. Dalmay, A. Griol, J. Hurtado, H. Bohlmann, R. Götzen, and J. García-Rupérez, "Experimental study of the evanescent-wave photonic sensors response in presence of molecular beacon conformational changes," J. Biophotonics **11**, 1–7 (2018).