Chapter 66
Aptamer Based Whispering Gallery Mode Biosensor

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Abstract Whispering gallery mode resonators (WGMR), in the form of optical silica microspheres, have been recently proposed as an efficient tool for the realisation of optical biosensors. A crucial step for producing reliable biosensors is their surface functionalization. In this work we report a functionalization process based on the use of a DNA aptamer. Also the optical characterization of the functional WGMR was performed, confirming that their resonance properties are little affected by the treatments. The characterization of the immobilized aptamer layers suggests that a suitable density of bio-recognition molecules is obtained.

Introduction

Microspherical whispering gallery mode resonators (WGMR) have inspired a number of proposed biomedical sensors [1], including thrombin detection [2]. WGMR are a valid alternative to surface plasmon resonators (SPR) [3]. Like SPR, WGMR are evanescent wave sensors that are capable of detection of single virions [4].

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Compared to SPR silica based WGMR have two important advantages: a significantly longer evanescent tail due to a reduced refractive index contrast and the wide variety of surface chemistry already established for silica. The combination of a high quality factor microsphere and a covalent chemistry for surface functionalization will rise to a very accurate generation of protein sensors.

We have tested the binding capabilities of two different thrombin DNA-aptamers and one VEGF DNA-aptamer. The 15-mer DNA-aptamer binds to the fibrinogen exosite and the 29-mer binds to the heparin exosite of thrombin protein. We have effectively immobilized all aptamer sequences and studied the functionalized resonator morphologically by means of atomic force microscopy and confocal laser scanning microscopy. Afterwards, we tested the performance of the WGMR aptasensors in a microfluidic flow cell under laminar flow conditions in buffer solutions and in 10 % non filtered human serum solution.

Experimental

Surface Functionalization

Microspheres can easily be fabricated directly on the tip of a standard telecom fiber. For this purpose we used a fiber fusion splicer (FITEL S182K). A cleaved tip of the fiber is inserted in one arm of the splicer and a series of arcs are then produced. The tip partially melts and surface tension forces produce the spherical shape. The size of the spheres increases with the number of arc shots, till it tends to saturate at a diameter of about 350 μm. The microspheres used in this work have an average diameter of 260 μm and stored under vacuum, in order to avoid contamination. After an activation in piranha solution (H_2SO_4:H_2O_2 4:1 v/v) for 3 min to remove organic contaminants and oxidize the surface, then rinsed in MilliQ water and dried at 110 °C for at least 2 h. The WGMR was immersed in a 3-mercaptopropyltrimeth oxysilane (MPTMS) toluene solution. Two different thrombin specific DNA-aptamers (15-mer, TBA-15, and a 29-mer, TBA-29), and one vascular endothelial growth factor (VEGF) specific DNA-aptamer were then immobilized on WGMR. In order to unfold the sequences strands and get the dithiol groups available for the immobilization reaction, a thermal treatment at 95 °C for 1 min was performed, followed by a thermal shock in ice for 10 min. The optical biosensor was prepared by incubating a 10 μM aptamer solution (in 0.5 M carbonate buffer pH 9) on previously silanized microspheres for 2 h using an orbital shaker, followed by a washing step in the same buffer. Finally, the samples were passivated in 1 mM MP-ET for 2 h at room temperature, reducing the aptamer density by a factor of 22. This process promotes the target recognition, allowing by one hand the aptamer structuration and

blocking by the other hand residuals free thiol groups. The silanization efficacy was evaluated morphologically by means of AFM (Atomic Force Microscopy) measurements. Using a fluorescent derivative of both aptamers, their immobilization was evaluated in terms of homogeneity using confocal microscopy analysis.

Experimental Setup

The laser light is coupled to the WGM resonator by means of a tapered fiber of about 3 μm diameter, produced in-house too. We used a microfluidic flow system that incorporates the WGMR and the tapered optical fiber. Our system consists of an open cell of 350 μL of volume with two entries connected to a peristaltic pump in an open loop configuration in order to constantly wash the excess of protein. The tapered fiber was bound to the bottom of the cell by UV adhesive and placed parallel to the flow. A typical experiment was run at 167 μL/min flow rate. Before the experimental measurements, the microfluidic flow system was passivated using filtered Prionex solution diluted 1:1,000 in buffer for 30 min and then rinsed with the same buffer for at least 10 min. This step was performed to avoid the protein adsorption on the fluidic cell. The microsphere was then slowly lowered into contact with the thinnest region of the taper and aligned at the centre of the flow cell. The light transmitted through the coupler-WGM resonator system was monitored at the output of the taper using an amplified InGaAs photodiode detector connected to an oscilloscope [5].

Results

The functionalization procedure was deeply investigated and the results were reported in our previous paper [6]. Two different aptamers for thrombin recognition, TBA-15 and TBA-29, were then immobilized onto the WGMR surfaces and the binding performance of each one was studied. We also performed two different control experiments, the first one consisted in injecting BSA of similar concentration into the flow cell with the aptamer immobilized on the WGMR surface. Adding BSA to the flow cell had minimal effect on the WGMR response for the aptasensors, indicating a good selectivity of both aptasensors to thrombin. We also tested the reusability of the WGMR sensor. The sensor regeneration was done by 50 mM NaOH solution and the WGMR aptasensor was then used for another detection cycle. Figure 66.1 shows that the binding capacity was maintained after two cycles of denaturation and equilibrium was reached after the first injection of 5 μl of 0.1 mg/ml of thrombin.

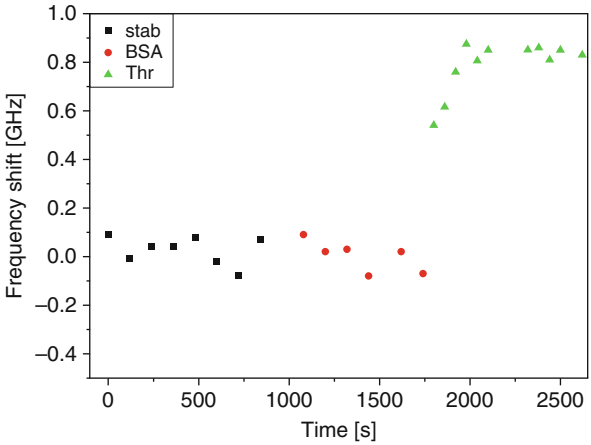

Fig. 66.1 Control experiment: Stability (*squares*), WGMR response of a TBA-15 sensing surface for 0.3 mg/ml BSA injected into the flow cell (*diamonds*) and Sensorgram of thrombin binding to a WGMR immobilized with TBA-15 in buffer, following the addition of thrombin (*triangles*)

Conclusions

We have demonstrated a WGMR biosensor aptamer-based detection of proteins, namely, thrombin and VEGF165. For thrombin we have studied the binding interactions of two DNA-aptamers (15-mer, TBA-15, and a 29-mer, TBA-29). We have developed an immobilization protocol for all aptamer sequences in such a way that the high Q factors (greater than 10^5 in buffer) are preserved. We have performed a series of morphological studies in order to assess the quality of the surfaces and determine whether the covalent binding is achieved. The data obtained from the AFM measurements are in good agreement with the optical ones. The covalently bound aptamers were found to form stable layers with sufficient number of binding sites demonstrating preserved recognition activity. Detection of thrombin in human serum was also performed, showing a very good specificity of WGMR sensors. WGMR aptasensors showed fast response, high specificity and good reversibility. It was also shown that WGMR sensors can be used for determining binding kinetics.

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