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Microfluidic platform based on a one-dimensional photonic crystal for label-free optical detection of oligonucleotides

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Received November 23, 2023
Revised November 23, 2023
Accepted November 28, 2023

Small circulating extracellular nucleic acids are one of clinically relevant classes of biomarkers of various human pathologies, including autoimmune diseases, cancer, and infectious diseases. To detect circulating DNA (cDNA) in liquid samples, the real-time polymerase chain reaction method based on amplification of cDNA molecules and determination of the degree of accumulation of the fluorescent signal of the reporter tag over time is used. The development of new alternative approaches for rapid and highly accurate detection of small-sized cDNA is of current interest. Optical biosensors for label-free detection based on the analysis of surface wave propagation changes on the surface of a one-dimensional photonic crystal can be adapted for oligonucleotide detection, and their combination with a microfluidic platform can miniaturize, and increase the performance of the bioanalytical procedures. In this study, we demonstrate the possibility of label-free optical detection of low-molecular-weight single-stranded DNA with different numbers of bases without additional signal amplification in the microfluidic mode using a biosensor based on a one-dimensional photonic crystal.

Keywords: One-dimensional photonic crystal, surface waves, label-free optical detection, single-stranded DNA, microfluidic platform.

DOI: 10.61011/EOS.2023.11.58052.5786-23

Introduction

The development of new approaches to rapid and high-precision detection of liquid analytes is an important trend in the field of clinical diagnostic practice [1]. The use of microfluidic technologies for this purpose makes it possible to effectively increase the detection productivity and miniaturize and automatize the analytical procedure by reducing the quantities of reagents used, specifically due to external equipment (a detector and peristaltic pumping systems) with minimal intervention of the user [2]. Methods for rapid label-free optical detection of liquid analytes are widely used in clinical diagnosis because they are applicable to a wide concentration range of analytes, do not require optical (fluorescent or chromogenic) labels, and can be adapted for multiplexed and high-throughput analysis [3].

For label-free detection of tumor markers [4], bacterial toxins [5] and other biomarkers [6], optical sensors based on one-dimensional photonic crystals can be used. Their multilayer structure characterized by periodic variation of the refraction index along one dimension ensures long-range propagation of optical waves over its surface, which provides ultrasensitive detection of biomolecules and recording of biomolecular interactions [7]. These optical biosensor platforms are versatile and allow the use of specific biorecognition molecules covalently bound to the sensor surface for high-precision detection of liquid analytes.

Circulating extracellular nucleic acids are an important class of diagnostic markers for autoimmune, oncological, and infectious human diseases [8]. The standard approach for detecting circulating DNA (cDNA) is real-time polymerase chain reaction (RT-PCR). However, due to the need to amplify and analyze the accumulation of the fluorescence signal of the reporter tag, the RT-PCR method is a time-consuming procedure. In addition, this method requires expensive equipment and special diagnostic kits [9]. Therefore, the development of alternative and versatile approaches for detecting nucleic acids, including adaptation of the existing optical detection methods for this purpose, is an urgent bioanalytical task. This study demonstrates the possibility of optical label-free detection of low-molecular-weight oligonucleotides with different numbers of bases referred to as single-stranded DNAs in the flow mode using a microfluidic platform and a biosensor for analysis of changes in the propagation of surface waves in a one-dimensional photonic crystal (SWPC).

Experimental part

Sensory system

For label-free optical detection, we used a sensor system based on a one-dimensional photonic crystal ~ 22 × 25 mm in size made of BK-7 glass with a sputtered coating of alternating layers of silicon oxide (SiO_2) and titanium oxide (TiO_2) as described previously [10]. The deposited layers formed a $TiO_2(SiO_2/TiO_2)_5SiO_2$ structure supporting a *p*-polarized surface mode at 500 nm that had an effective refraction index of 1.38. The thicknesses of the titanium oxide and silicon oxide layers were 64 and 214.3 nm, respectively, and the thickness of the outer silicon oxide layer was 298.8 nm. Detection of the interaction of model oligonucleotides of various lengths with the surface of a photonic crystal was carried out by imaging the preliminarily biofunctionalized surface of the photonic crystal after its mounting on the surface of a prism in the Kretschmann configuration. In this case, the excitation wavelength of the photonic crystal was 500 nm, which corresponded to an intermediate position between the maxima of the blue (~ 460 nm) and green (~ 545 nm) pixels of the color camera recording changes in the image of the surface of the photonic crystal.

Excitation of a photonic crystal at the given wavelength typically results in the appearance of a resonance peak. Shifts of the position of the resonance peak of the photonic crystal related to the detected intermolecular interactions on the sensor surface were taken into account by analyzing changes in the intensities of both pixels and subsequent conversion of changes in their intensities into changes in the thickness of the molecular layer on the sensor surface [10].

The described changes were presented in the form of sensorgrams reflecting the changes in the thickness of the molecular layer on the sensor surface with time upon the introduction of working buffer solutions and solutions of model single-stranded DNAs interacting with recognition elements chemically immobilized on the surface of the photonic crystal.

Biofunctionalization of the surface

To biofunctionalize the photonic crystal using chemical covalent binding of recognition elements, its surface was first modified by incubation in a 1% solution of (3-aminopropyl)triethoxysilane (Merck Group, France) in acetone (Acros Organics/Thermo Fischer Scientific, France). Then, the surface of the photonic crystal was activated by incubation in a 2.5% solution of glutaraldehyde (Merck Group, France) in 0.02 M phosphate-buffered saline (PBS), pH 7.4, for 60 min. After thoroughly washing the surface with 0.02 M PBS, pH 7.4, 1-μl aliquots of working solutions of neutravidin (NA, Thermo Fischer Scientific, France) in 0.02 M PBS, pH 7.4, with concentrations of 1.0, 1.5 and 2.0 mg/ml were applied onto the activated surface of the photonic crystal using a dispenser and incubated for 5 min. Excess ND solution was removed from the surface of the photonic crystal by blowing, and the substrate was mounted on the surface of the prism.

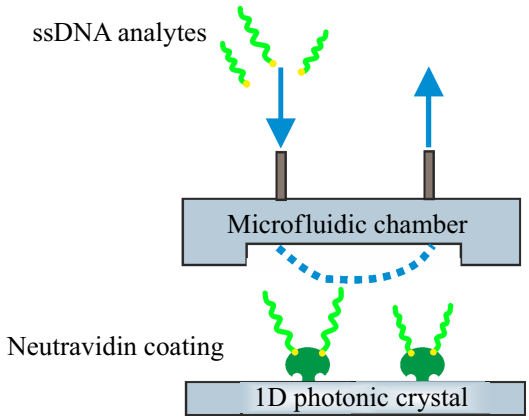

Figure 1. Schematics of the detection of model single-stranded DNAs in a flow using a microfluidic platform and a sensor based on a one-dimensional photonic crystal. The arrows indicate the directions of injection and removal of the analyte from the microfluidic platform. Designations: ssDNA, single-stranded DNA; 1D, one-dimensional.

Maximum increase in the average thickness of the sensor surface after successive injections of solutions of model analytes, biotinylated single-stranded DNAs containing different numbers of bases

Analyte	Mean da_{max}, nm		
	Density of sensor coating with neutravidin:		
	1.2 mg/mm^2	1.8 mg/mm^2	2.4 mg/mm^2
Single-stranded DNA (50 bases)	0.43601	0.39985	0.29696
Single-stranded DNA (30 bases)	0.34374	0.30351	0.23526

Microfluidic platform

To detected analytes, a microfluidic cell was used that had two holes (inlet/outlet) and metal connectors for fixing tubes with an internal diameter of 0.51 mm and passing analyte solutions in the flow mode using a three-channel peristaltic pump (Ismatec Reglo ICC, USA) controlled by a computer. The microfluidic cell had one channel with a depth of 5–50 μl and a volume of 0.5–5 μl. After assembling the microfluidic platform, the microfluidic cell was filled with a working buffer solution (0.02 M PBS, pH 7.4) at a rate of 30 μl/min. After the cell was filled, the solutions were injected at a flow rate of 30 μl/min in the following orfder: working buffer solution (0.02 M PBS, pH 7.4), 600 s; blocking buffer solution (2 mg/ml

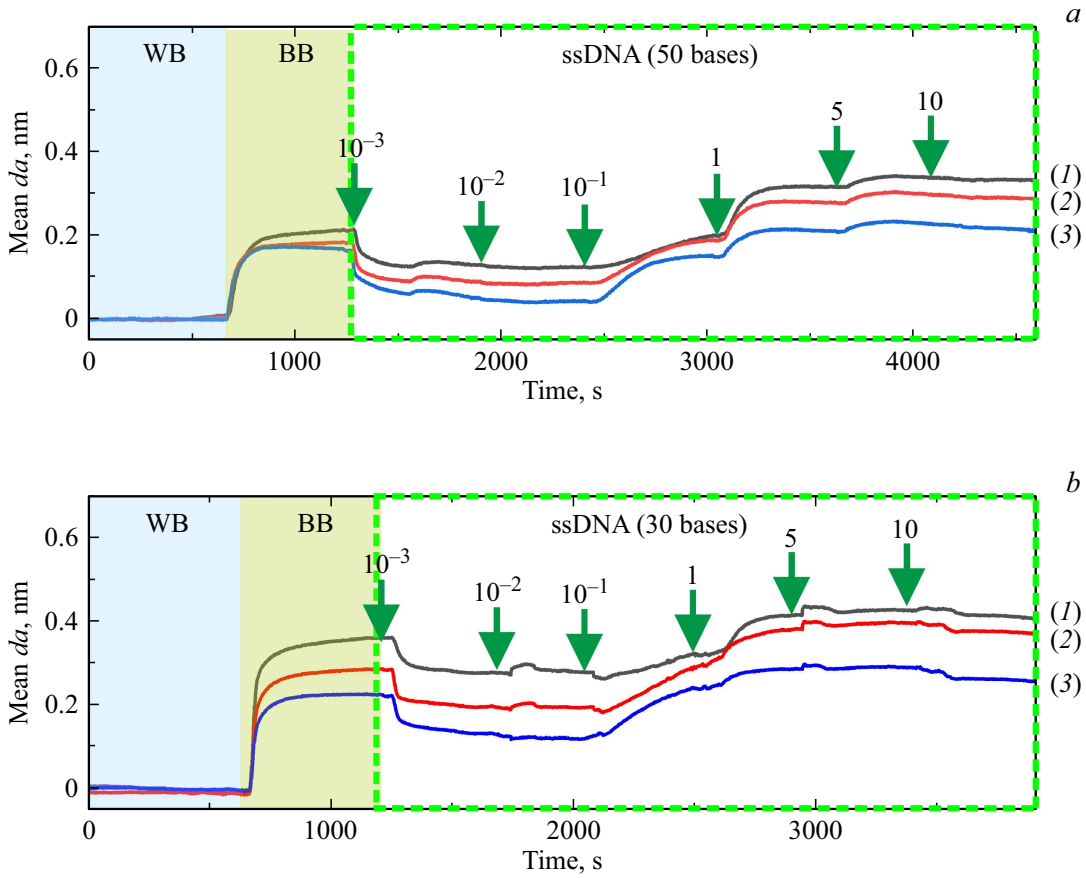

Figure 2. Sensorgrams obtained as a result of detection of biotinylated single-stranded DNAs containing sequences of (*a*) 50 or (*b*) 30 nucleotide bases with the use of a one-dimensional photonic crystal whose surface was functionalized with the biotin-binding protein neutravidin. The arrows indicate the start of the injection of oligonucleotide solutions. The numbers indicate the concentrations of the oligonucleotide solutions (μM). Designations: WB, working buffer; BB, blocking buffer; ssDNA (50 bases), single-stranded DNA containing 50 nucleotide bases; ssDNA (30 bases), single-stranded DNA containing 30 nucleotide bases. The numbers on the right indicate sensorgrams obtained using photonic crystals with different densities of the coating of their surface with neutravidin: *1*, 1.2 μg/mm^2, *2*, 1.8 μg/mm^2, *3*, 2.4 μg/mm^2. Each curve is an average of three sensorgrams.

solution of bovine serum albumin (BSA) in 0.02 M PBS, pH 7.4), 600 s; 0.001–10 μM solutions of model single-stranded DNAs containing 30 or 50 thymidine bases and biotinylated at the 5′ end (Eurofins Genomics, Germany) in 0.02 M PBS, pH 7.4; and working buffer solution (0.02 M PBS, pH 7.4), 400–600 s. All solutions used for injection into the microfluidic system were previously degassed using an ultrasonic bath (Elma Sonics, Germany).

Results and discussion

As a result of preliminary activation and biofunctionalization, the biotin-binding recombinant protein NA was chemically immobilized on the surface of the photonic crystal. Before its application, the sensor surface was aminosilanized and activated with glutaraldehyde to ensure effective binding of the protein molecules. Primary amino groups in the surface layer of APTES react with glutaraldehyde to form Schiff bases, which allows chemical binding of NA through covalent binding of primary amino groups in the amino acid sequence of the protein to the sensor surface [11]. Due to the specific structure of NA, its covalent binding to the surface of a photonic crystal ensures steric accessibility in the microfluidic regime for at least 2 out of 4 available binding sites for the biotinylated analyte [12] (Fig. 1).

As a result of using NA solutions with different concentrations in the reaction, the efficiency of covalent binding of NA to the sensor surface was 96.2 ± 0.5%. After installing the biofunctionalized sensor into the microfluidic platform, working buffers and solutions of the studied analytes were introduced in the flow mode until the signal from the sensor reached a plateau. To reduce nonspecific binding, the sensor surface was treated with a blocking buffer in the flow-through mode, after which successive injections of solutions of the model biotinylated single-stranded DNAs were carried out until the signal from the sensor reached a plateau (Fig. 2).

The obtained sensorgrams show a substantial increase in the sensor signal upon the introduction of $0.1\,\mu$M solutions of both types of oligonucleotides for all analyzed densities of the sensor surface coating with the recognition element (NA). In this case, the maximum signal saturation indicating the maximum possible binding of analytes to the surface was observed upon the introduction of $1-10\,\mu$M solutions of both types of oligonucleotides. The table show the values of the maximum increase in the thickness of the sensor surface layer in analyzing the binding of both types of analytes after a complete titration cycle.

With increasing concentration of the NA solution used to modify the surface of the photonic crystal, the maximum increase in the thickness of the sensor surface layer (Mean $da_{\max}$) was decreased for both types of oligonucleotides, which may have resulted from aggregation of this protein on the sensor surface with an increase in its content in the reaction mixture upon biofunctionalization leading to the formation of a multilayer protein coating. This effect makes it more difficult to recognize biotinylated substrates, in particular, oligonucleotides, with a higher molecular weight (50 bases; molecular weight, ~ 15.5 kDa) compared to those with a lower molecular weight (30 bases; molecular weight, ~ 9.2 kDa) [13].

Conclusion

The data obtained in this study demonstrate the possibility of detecting low-molecular-weight DNAs with different numbers of bases in a flow mode using a microfluidic platform and a label-free optical sensor based on a one-dimensional photonic crystal. Thus, chemical modification of the photonic crystal surface provides effective immobilization of the recognition element of the sensor, the biotin-binding protein NA, which ensures reliable detection of low-molecular-weight single-stranded DNAs labeled with biotin at a minimal concentration of $0.1\,\mu$M without additional signal amplification. The label-free optical detection microfluidic platform developed here can be subsequently adapted for the detection of small extracellular circulating nucleic acids in liquid clinical samples.

Funding

This study was supported by the Ministry of Science and Higher Education of the Russian Federation, grant No. 075-15-2021-935.

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by E.Potapova