DEVELOPMENT OF A MICROFLUIDIC BIOSENSOR FOR THE DIAGNOSTICS AND TYPING OF *MYCOBACTERIUM TUBERCULOSIS*

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Background: *Despite the general trend towards decreasing the incidence of newly diagnosed active forms of tuberculosis, the situation with this disease spread in the Russian Federation remains extremely tense. At the same time, the diagnosis is performed according to the standard scheme, which takes about a month; another month is spent on the drug sensitivity tests. Thus, the development of new methods for the diagnostics and typing of mycobacteria, as well as their practical implementation is an urgent problem. The modern approaches in the field of microfluidic technologies open great opportunities in this direction.* ***Aims:*** *Development of a method for identification and typing of Mycobacterium tuberculosis using a label-free biosensor based on surface waves in a one-dimensional photonic crystal (PC SM biosensor).* ***Methods:*** *Oligonucleotide probes were selected and synthesized as DNA targets for M. tuberculosis typing. The photonic crystal surface was modified with aqueous solutions of (3-aminopropyl)triethoxysilane, Leuconostoc mesenteroides dextrans and bovine serum albumin. The experiments were carried out using a PC SM biosensor.* ***Results:*** *The sequences of detecting oligonucleotide probes were selected for spoligotyping of M. tuberculosis using the PC SM biosensor. Their 3′-ends were modified in order to create extended single-stranded regions which are not subjected to the formation of secondary structures and facilitate hybridization with a single-stranded DNA target. Several series of experimental PC surface modifications were carried out using L. mesenteroides dextrans with different functional groups (including real-time detection of the modification results). The simultaneous registration of the increment layer size and volume refractive index of the mixture excludes the use of a reference cell. Besides, the experiments were carried out to detect the specific binding of biotinylated oligonucleotide probes to the modified PC surface.* ***Conclusions:*** *A technique for the design of probes has been developed and a model system of oligonucleotides for the detection of single-stranded DNA using a PC biosensor has been proposed. The developed technique for the PC surface modification with dextrans from L. mesenteroides allows increasing the sensitivity of oligonucleotides detection using the PC SM biosensor. This approach will further expand the panel of diagnostic probes, including those used for the identification of resistance markers.*

Keywords: *biosensor; tuberculosis; Mycobacterium tuberculosis; microfluidic technologies; biochips; photonic crystal surface modes; photonic crystal; personalized medicine.*

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List of abbreviations

PCSW-biosensor, Photonic Crystal Surface Waves Biosensor — a complex device for detecting the processes of sorption/desorption of biomolecules on a specific sensitive surface.

1D PC — One-Dimensional Photonic Crystal: a special synthetic surface, which is a multilayer dielectric spectrally selective structure.

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BACKGROUND

According to the latest data from the World Health Organization, the incidence of tuberculosis in the Russian Federation continues to remain at a high level, despite the emerging trend towards stabilization. In 2019, more than 60 thousand new cases of the disease and 7536 deaths were registered [1, 2]. A special and very significant role in the exacerbation of the situation with tuberculosis is played by the emergence and increasing distribution of drug-resistant strains of mycobacteria. According to some data, in the world about 4% of tuberculosis cases are caused by strains with multidrug resistance (MDR), and among previously treated patients, the frequency of detection of MDR strains reaches 40%. At the same time, on the territory of the Russian Federation, the indicators are even more negative. Thus, the number of MDR strains among newly diagnosed cases reaches 30%, while among treated patients it exceeds 60% in other regions [2, 3]. Thus, in the near future, modern medicine, devoid of effective anti-tuberculosis drugs, may be powerless against the growing threat of the widespread spread of resistant strains of *Mycobacterium tuberculosis*.

Obviously, in this situation, research aimed at solving the problem of rapid diagnosis of the disease, timely detection of anti-TB drugs-resistant forms, as well as adequate anti-epidemic measures aimed at preventing the spread of mycobacterial strains (including resistant ones) in the human population, are very relevant. Modern advances in molecular biology and biophysics open up great prospects and opportunities in this direction. In particular, developments in the field of microfluidic technologies and optical markerless biosensors are fundamentally new. The use of a microfluidic markerless biosensor on surface optical waves in a one-dimensional photonic crystal (PCSW-biosensor) makes it possible to significantly simplify the molecular identification of pathogens in laboratory diagnostics of infections, including tuberculosis. The PCSW-biosensor makes it possible to analyze a wide range of interactions: from the formation of various protein-protein complexes to the interaction of oligonucleotides of different sequences. The main advantage of the technology is the passage of reactions in an isolated zone of minimum volume, which eliminates contamination, reduces the analysis time, and makes the analysis procedure convenient for the operator. Registration of such interactions is carried out in real time and does not require preliminary labeling of target biomolecules [4]. In 2020, the multiplex potential of a PCSW-biosensor with a two-dimensional spatial resolution was shown [5]. Thus, the urgency of using this technology when working with the causative agent of tuberculosis is obvious.

The aim of the study was to develop a method for the identification and typing of *M. tuberculosis* using a microfluidic PCSW-biosensor.

METHODS

Probes

The collection of genome-wide sequences for 5721 *M. tuberculosis* samples was used to select probes that provide family-specific typing of the tuberculosis pathogen. For spoligotyping, specific spacer sequences of the DR region (direct repeat) were determined in the genome of M. tuberculosis strain H37Rv (NCBI Reference Sequence: NC_000962.3). Based on previously published data [6], the sequences of probes providing primary typing of M. tuberculosis for 43 DR-region spacers were determined. In the presented work, several nucleotides were added to the 3'end of DNA probes, allowing the site of hybridization with single-stranded DNA of the spacer to be increased to 20 base pairs. The terminal 3'-terminal nucleotide of the probe is modified with biotin for immobilization on streptavidin. The nucleotide sequence of the obtained DNA probe for the detection of the 43rd spacer: 5'-GGAGGTGCAGCA-acgtatac-3'-Biotin. The control of the secondary structure of a single-stranded DNA probe and its potential target was carried out using the mFold program [7].

Biosensor

The registration of the process of interactions of biomolecules on the surface was carried out in real time using a microfluidic marker-free PCSW-biosensor "EVA 2.0" (Russia) [4, 8]. The sensitive surface of the biosensor is the final silicon oxide layer of a one-dimensional photonic crystal (1D PC). To modify the surface of the photonic crystal, we used aqueous solutions of (3-aminopropyl) triethoxysilane ([3- (Aminopropyl) triethoxysilane], APTES), Leuconostoc mesenteroides dextrans, bovine serum albumin, polyallylamine, glutaraldehyde, epichlorohydrin phosphate -buffered saline, PBS).

RESULTS

The design of oligonucleotide probes for the detection of the causative agent of tuberculosis has been developed, their optimal length has been selected for hybridization with the target DNA. To increase the detection sensitivity in experiments with oligonucleotide

DNA targets, it was initially required to increase the sorption capacity of the photonic crystal surface, which was achieved by creating a branched polysaccharide structure on its surface. In this work, we used dextran with a Mw of 500 kDa, which was previously chemically modified to introduce available functional groups into the polymer chain for binding to the silanized surface of the 1D OC.

The kinetics of the interaction of the model bovine serum albumin protein with the modified surface was recorded on a PCSW-biosensor; for comparison, the sorption curves are shown for the 1D OC surface modified by two methods: silanization with APTES and modification with polyallylamine and glutaraldehyde [9] (Fig. 1). Compared to the APTES-modified 1D OC surface, the sorption capacity of the surface with the polysaccharide increased by 20%, which can be explained by the appearance of a three-dimensional branched structure of dextran chains.

Optimal conditions for the modification of the surface of the 1D OC with dextran were selected, which make it possible to achieve a higher sorption capacity of the surface of the 1D OC for the subsequent sorption of low-molecular-weight ligands. Sorption curves for the detection of oligonucleotide DNA targets simulating unique regions of the *M. tuberculosis* genome were obtained using a microfluidic markerless PCSW-biosensor. The sensitivity of the technique is determined by the signal-to-noise ratio when registering the process of binding of oligonucleotides to the surface of the OPC of the biosensor. Our results show a sensitivity of 0.7 pg/mm^2.

For the covalent binding of biotinylated oligonucleotide DNA probes in real time on the surface of the 1D OC and subsequent registration of the specific interaction of the probes on the activated surface of the 1D OC, streptavidin was preliminarily immobilized. Nonspecific binding sites on the 1D OC surface were blocked by 0.1 mg/ml bovine serum albumin solution (Fig. 2).

DISCUSSION

Today, the problem of rapid and early diagnosis of tuberculosis is relevant all over the world. Work is underway to identify proteins of mycobacteria in human blood [10]. However, a systematic approach to the study of the physiology of mycobacteria showed the limitations of such methods [11]. The nucleotide sequences of the probes selected in this study correspond to the spacers located between the direct repeats (DR-regions) of the *M. tuberculosis* genome, and provide reliable typing of the pathogen by belonging to the family [6]. It was previously shown that the method of multiplex biosensing on the surface waves of the 1D OC with two-dimensional spatial resolution in real time can be used to register antigen-antibody binding processes with sufficient sensitivity [12], and therefore, in this work, the method was adapted for typing the causative agent of tuberculosis. The method for modifying a photonic crystal proposed in this work provides a higher adsorption capacity of the surface

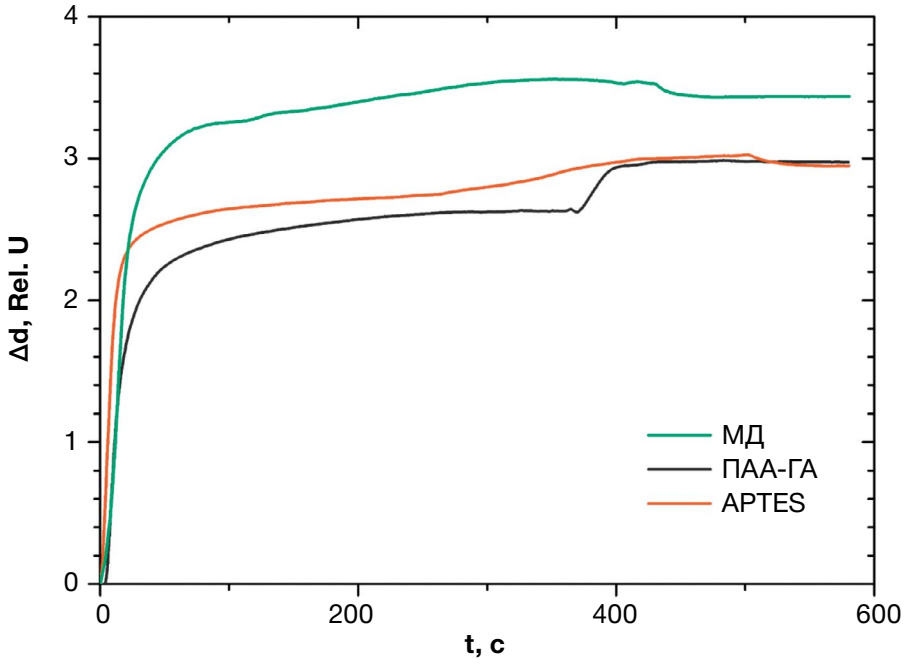

Fig. 1. The change in the adsorbed layer thickness (Δd) upon bovine serum albumin binding to the modified PC surface.
Note. МД — modified dextran; ПАА — polyallylamine; ГА — glutaraldehyde; APTES — 3-(Aminopropyl)triethoxysilane.

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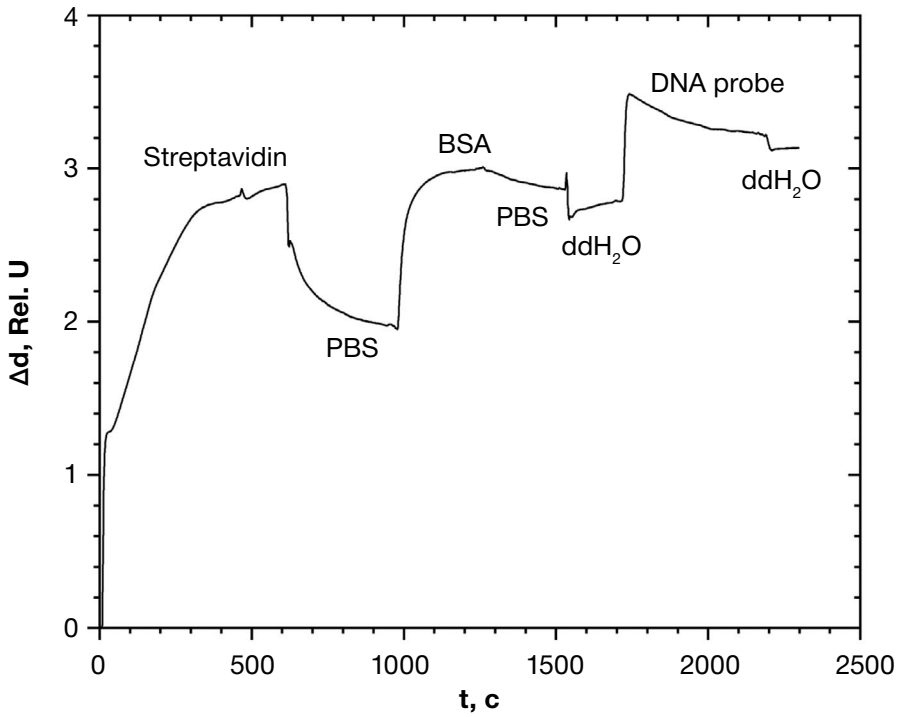

Fig. 2. Registration of the sequential adsorption of streptavidin and a biotinylated DNA probe, 5'-GGAGGTGCAGCA-acgtatac-3'-Biotin, on the modified PC surface.

Note. PBS — phosphate-buffered saline; БСА — bovine serum albumin.

than those known from the literature [9] and allows one to solve the problem of sensitivity associated with a small number of bacteria in a biological material. In addition, a potential solution to this problem can be the implementation of a certain optimal number of cycles of the polymerase chain reaction before detection on the PCSW-biosensor.

Thus, on the basis of the study carried out, it can be concluded that the diagnostic system based on the PVFC-biosensor has a great potential for rapid diagnosis and typing of the causative agent of tuberculosis.

CONCLUSION

In the course of the work, we analyzed the modification of the photonic crystal surface based on the interaction of the activated surface with the functionalized dextran *L. mesenteroides*. A method for the design of probes was developed and a model system of oligonucleotides for the detection of single-stranded DNA of the tuberculosis pathogen using a microfluidic PCSW biosensor was proposed. This method of modifying and activating the working surface of a photonic crystal makes it possible to bind oligonucleotides of different sequences for further use of the PCSW-biosensor in diagnostics.

The proposed approach will further expand the panel of diagnostic probes, including for the identification of resistance markers. In turn, the use of such a biosensor in the diagnosis of tuberculosis infection will ensure the fastest possible diagnosis, determination of drug resistance of strains and, accordingly, the appointment of a treatment regimen as soon as possible.

ADDITIONAL INFORMATION

Authors contribution. Basmanov D.V., Bespyatykh J.A. — study concept, manuscript writin; Mitko T.V., Shakurov R.I., Shirshikov F.V., Sizova S.V., Alieva E.V., Konopsky V.N. — data acquisition and analysis, literature review, manuscript writing. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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Competing interests. The authors declare that they have no competing interests.

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