NANOWIRE-BASED BIOSENSORS FOR SOLVING BIOMEDICAL PROBLEMS

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The review considers modern achievements and prospects of using nanowire biosensors, principles of their operation, methods of fabrication, and the influence of the Debye effect, which plays a key role in improving the biosensor characteristics. Special attention is paid to the practical application of such biosensors for the detection of a variety of biomolecules, demonstrating their capabilities and potential in the detection of a wide range of biomarkers of various diseases. Nanowire biosensors also show excellent results in such areas as early disease diagnostics, patient health monitoring, and personalized medicine due to their high sensitivity and specificity. Taking into consideration their high efficiency and diverse applications, nanowire-based biosensors demonstrate significant promise for commercialization and widespread application in medicine and related fields, making them an important area for future research and development.

Key words: biosensor; silicon; nanowires; biomedicine; diagnostics

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INTRODUCTION

Biosensors are analytical devices that consist of a biological recognition element (e.g., oligonucleotides, aptamers, antibodies) and a physicochemical transducer, which converts the biological interaction into an electrical, optical, or other signal. Biosensors play an important role in medical diagnostics, environmental monitoring, and food safety. Among different types of biosensors, nanowire biosensors (NW biosensors) attract much interest due to the high sensitivity of analysis and convenient sample preparation, which does not require analyzed substance labeling.

In NW biosensors, nanowires (or nanoribbons), made of various materials, such as silicon, indium phosphide, or gallium nitride, are used as the physicochemical converter [1]. The surface area of the sensor is usually very small (micrometers), but it is possible to use a sufficient volume of analyte (up to a milliliter), which makes it possible to change directly the electrical or optical properties of the sensor binding to a target molecule from the volume. NW biosensors can perform highly sensitive label-free detection of biomarkers, which makes them highly effective in applications requiring real-time measurements [2]. In addition, the surface of nanowires can be easily modified with a biological recognition element thus allowing detection of a wide range of chemical and biological substances [3].

Taking these advantages into consideration, it is clear that NW biosensors have a significant potential for the use in medical diagnostics. For example, NW biosensors can ensure early detection of oncological and infectious diseases by detecting specific biomarkers; this is critical for timely intervention and improving patient outcomes. In addition, their applicability for testing at the point of medical care and the possibility of creating portable devices based on them open new prospects for continuous health monitoring and personalized medicine.

In recent years, the direction of creating various biosensors especially their applications in the field of laboratory diagnostics has been actively developing in Russia. For example, the research and production company “Biosensor AN” [4] is involved in the production of express diagnostic test strips for determining various urinary and blood components; glucometers are actively used to determine and monitor blood glucose levels, and a number of companies have developed express diagnostic test strips for coronavirus detection.

Currently, biosensors are actively developed to detect various disease markers. For example, the Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences (IMB RAS) developed the TB-BIOCHIP test system to detect tuberculosis [5]. A detector for recording glucose and lactate (“Rusens”) was developed on the basis of the Laboratory of Electrochemical Methods of the Chemistry Faculty of Moscow State University [6]. Such microchips have found wide application in many Russian centers.

In Russia a unique NW biosensor has been developed jointly by teams from the Institute of Biomedical Chemistry (IBMC), the Institute of Semiconductor Physics (ISP SB RAS, Novosibirsk), NZPP Vostok (Novosibirsk), and Riko-Med (Moscow). Using the biosensor it is possible to detect target molecules using special chips; the manufacturing

technology for these chips is compatible with CMOS technology (CMOS is a complementary metal-oxide-semiconductor structure — a standard technology for industrial manufacturing of microcircuits). The chips contain an array of silicon nanowires based on silicon-on-insulator (SOI) structures in the sensor area. The operating principle of the NW biosensor is based on recording the current flowing through the nanowires.

Adsorption of a biological molecule on the surface of a nanowire is accompanied by changes in the surface potential. For biospecific analysis, the surface of nanowires is sensitized with molecular probes: antibodies, oligonucleotides, or aptamers. In this case, probe/target molecule complexes are formed on the surface of the nanowire due to affinity interaction. The binding event is recorded by the electronic system of the NW biosensor, and allows the target molecule to be identified in the analyzed material with high sensitivity. Using such NW biosensor the research group headed by Professor Yu.D. Ivanov (Laboratory of Nanobiotechnology of IBMC) has demonstrated the possibility of detecting nucleic acids and proteins with subfemtomolar sensitivity [7–11].

SOI chips are also used by other Russian research teams. For example, using SOI nanowire structures, the research group headed by Doctor of Physical and Mathematical Sciences V.A. Krupenin (Moscow State University) developed a method for recording changes in the time dependence of current at different pH values of buffer solutions [12]. The group guided by Professor D.V. Pyshny has proposed a new type of modification of the surface of SOI structures for highly specific detection of short RNA with femtomolar sensitivity. This modification consists of forming an ultrathin sensor-probe transition layer by means of carbonyldiimidazole or glycidoxypropyltrimethoxysilane [13].

At present, despite the active use of SOI chips and NW biosensors by Russian research teams, no Russian test systems based on NW biosensors have been registered in government agencies. One of the reasons is the complex, long and labor-intensive process of registering diagnostic equipment.

This review considers current achievements and prospects for the use of NW biosensors.

1. NANOWIRE BIOSENSOR

Highly sensitive and reliable biosensors with a high degree of reproducibility can be used both to detect various diseases at early stages and to monitor disease progression and determine treatment options. Since their development, biosensors are aimed to provide accurate, reproducible, and sensitive detection of target molecules in real time. However, in order to make biosensors available to a wider range of consumers and integrate them into clinical diagnostics, they must also be cost-effective.

Currently, silicon nanowires-based biosensors (Si-NW biosensors) are of great interest, as they have characteristics that ensure high sensitivity, biocompatibility, and stability of the device [14–16]. An additional advantage of silicon nanowires is the possibility of their surface functionalization, allowing realization of various biospecific approaches for recognition of biomolecules, which significantly expand the capabilities of the biosensor itself [17, 18]. In addition, using silicon nanowires-based biosensors, it is possible to perform fast, label-free, and continuous analysis in real time, which requires the use of small amounts of biological material [19]. Thus, Si-NW biosensors have the potential for practical application, especially in the field of clinical diagnostics, such as early detection of cancer.

Si-NW biosensors are typical field-effect transistor-based devices that contain such elements as a source, drain and gate electrode. The drain and source are connected by a semiconductor channel made of silicon nanowire. The source-drain current is regulated by changing the voltage on the gate electrode. In this case, the silicon nanowire is a sensor element, whose characteristics change in response to a change in the external electric field due to autoemission and electron transport [20]. The diagram of the Si-NW biosensor is shown in Figure 1.

The operating principle of the Si-NW biosensor is shown in more detail in Figure 2: negatively or positively charged target molecules bind to recognition elements (molecular probes) immobilized on the surface of a silicon nanowire, which changes the conductivity of the nanowire. Antibodies, aptamers, and oligonucleotide sequences can be used as molecular probes; by forming complexes with target molecules, they provide specificity of the analysis. Si-NW biosensors can have n- and p-type conductivity. Nanowires, in which unbound electrons act as charge carriers, are called n-type nanowires, and nanowires, in which the charge is transferred by “holes”, created by electron vacancies in ionic bonds inside the crystal lattice, are called p-type nanowires. The conductivity of the nanowire determines the differences in signal registration: for n-type Si-NW biosensors, the adsorption of negatively charged target molecules leads to a decrease in the conductivity of the silicon nanowire, while for p-type Si-NW biosensors it leads to an increase in conductivity.

The conductivity of silicon nanowires is significantly affected by the counterion condensation effect: negatively charged target molecules are surrounded in solution by positively charged counterions due to electrostatic interactions [21]. In this case, the Debye radius is a quantitative characteristic of the field effect, describing the depth of field penetration into the semiconductor. At the Debye radius, negatively charged molecules become electrically neutral, since the effect of negative

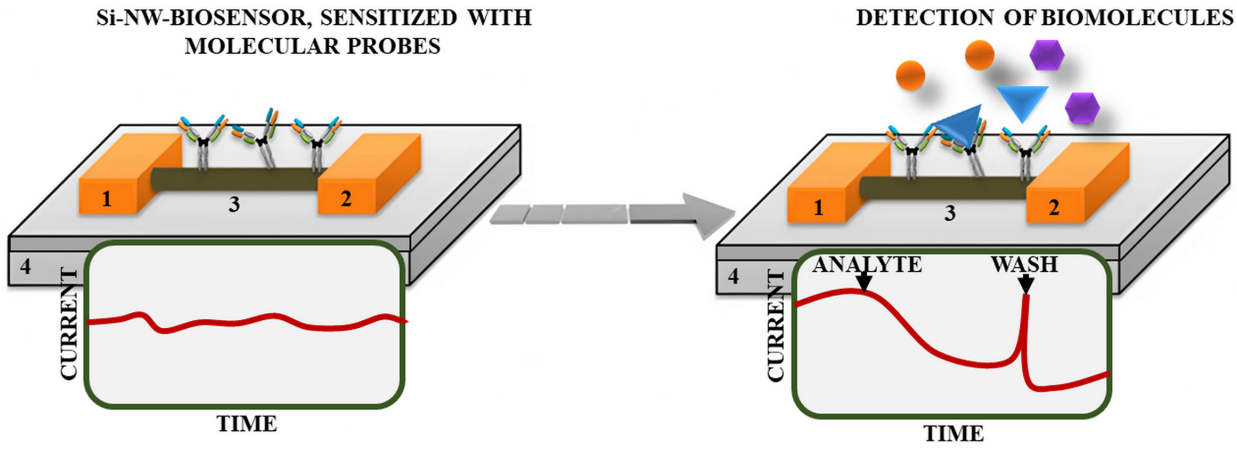

Figure 1. The schematic diagram of Si-NW biosensors and their principle of operation, where 1 – source electrode, 2 – drain electrode, 3 – semiconductor channel (nanowire), 4 – gate electrode.

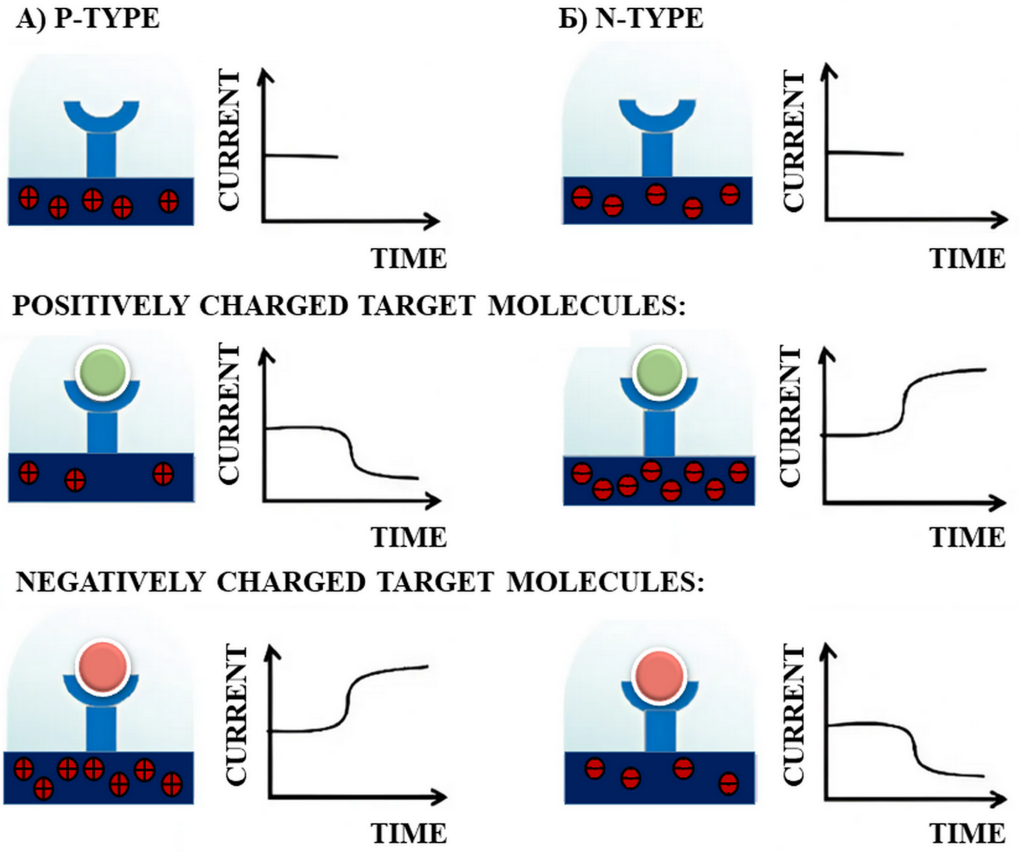

Figure 2. Working principle of Si-NW biosensors: **A)** p-type Si-NW biosensor; **B)** n-type Si-NW biosensor. The conductivity of the nanowire changes with time for different charged target molecules: **A)** in the case of p-type Si-NW biosensor, when a positively charged target molecule binds to the molecular probe, the conductivity decreases, when a negatively charged target molecule binds, the conductivity increases; **B)** in the case of n-type Si-NW biosensor, when a positively charged target molecule binds to the molecular probe, the conductivity increases, when a negatively charged target molecule binds, the conductivity decreases.

charges is compensated by positive charges arising from the electrostatic interaction of molecules, and does not lead to changes in the conductivity of the nanowire. Consequently, only target molecules within the Debye radius contribute to the change in conductivity. With a Debye radius of ~1 nm (for a solution with an ion concentration of 100 mM), the charges of the target molecules can be electrically detected on the sensing surface [22]. Typically, under physiological conditions and in highly concentrated solutions, the Debye length is small (~0.7–1 nm) [23]. A common method to reduce the Debye effect is to dilute the studied sample with a buffer solution with a low salt concentration to reduce

the ion concentration [24]. Stern et al. have shown that the sensitivity of the sensor is significantly improved in an electrolyte with a low ionic strength compared to a solution with a high ionic strength [25]. Figure 3 shows the relationship between the Debye length and ionic strength according to Stern et al.

Among the methods for fabrication of silicon nanowires, two main approaches can be distinguished: "top-down" and "bottom-up" [26]. In the bottom-up approach, silicon nanowires are grown on a bulk silicon substrate using chemical vapor deposition [16, 27]. The advantage of this approach is the ability to dope silicon nanowires directly during their growth by adding dopant precursors in the synthesis process, which allows for a more uniform dopant distribution and improved electrical properties of the nanowires [28]. However, this method requires expensive specialized equipment and subsequent processes for cleaving, aligning, and annealing the nanowires, which also increases the overall complexity of fabrication [28–30].

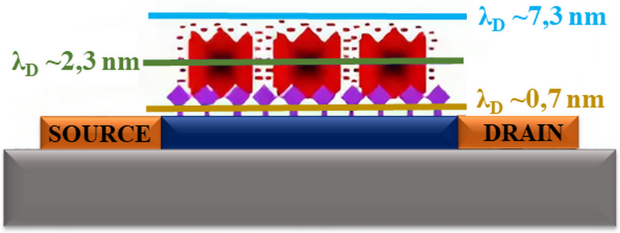

Figure 3. The Debye length model (λ_D) for different ionic strength of the sodium phosphate buffer (SPB) developed by Stern et al. The studied molecule is negatively charged streptavidin and the sensor is functionalized with biotin (rhombuses). The Debye length of 0.01 M SPB (λ_D ~0.7 nm), 1 mM SPB (λ_D ~2.3 nm), and 0.1 mM SPB (λ_D ~7.3 nm) are presented. The figure is adapted from [25].

The top-down approach, schematically shown in Figure 4, refers to the formation of arrays of bulk silicon nanowires by combining lithography techniques such as electron beam lithography and standard etching techniques to form bulk silicon nanowires [28]. It should be noted that electron beam lithography is one of the most common advanced lithographic processes used in the fabrication of Si-NW biosensors, which allows the processing of high-resolution nanostructures with high flexibility due to mask-free pattern formation [31]. The top-down approach allows fabrication of both horizontal silicon nanowires stacked on a silicon substrate and vertically aligned nanowires arranged perpendicular to the substrate, and provides well-oriented nanowires with high resolution and desired properties [28, 32]. The top-down approach also has the advantage of better contact integration and compatibility with the complementary metal-oxide-semiconductor (CMOS) fabrication process, as compared to the bottom-up approach. However, despite these advantages, the top-down approach requires the use of specialized equipment, which limits its application to laboratory experiments and prevents the widespread commercial use of Si-NW biosensors [33, 34].

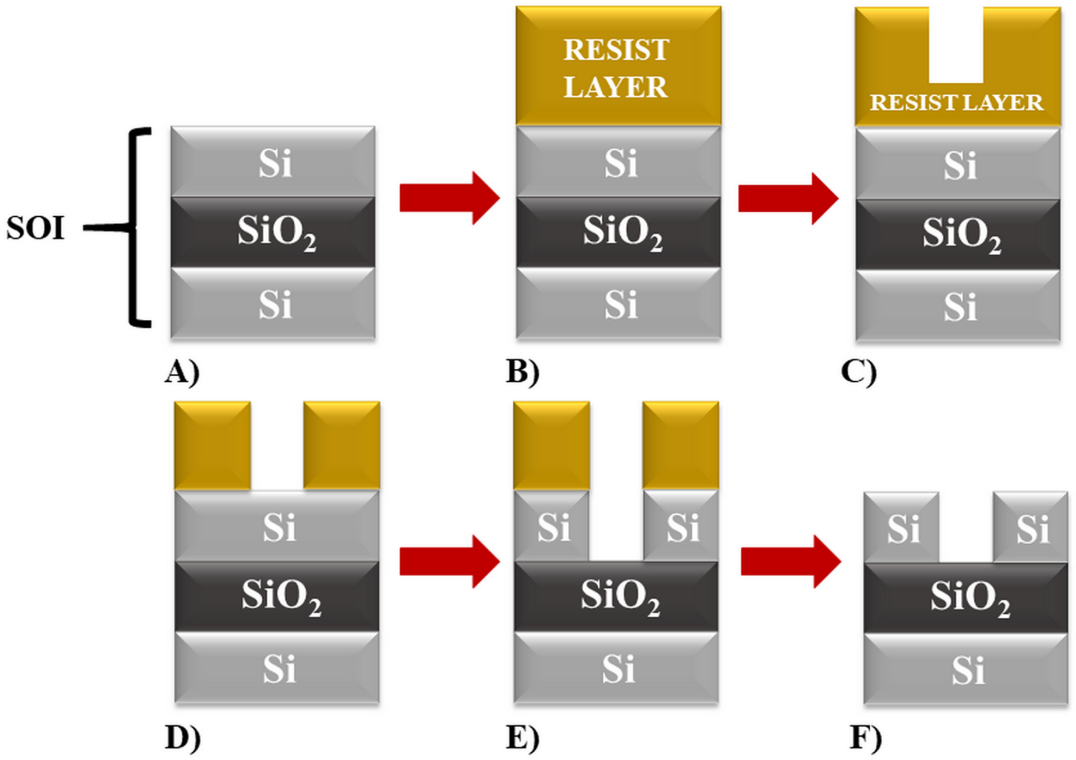

Figure 4. The scheme of the technological process of silicon nanowires fabrication using electron beam lithography, where **A)** SOI-substrate; **B)** spin coated resist layer; **C)** electron beam exposure; **D)** resist layer development; **E)** silicon etching; **F)** complete of the Si-NW fabrication process and removal of the resist layer.

Thus, the development of a commercial Si-NW biosensor remains at a preliminary stage and continues to face several challenges:

1. Analysis of complex biological samples, such as whole blood or urine. The presence of various compounds in these samples may lead to non-specific binding and reduced detection accuracy of target molecules.

2. Modification of the biorecognition layer. Reversible surface modification technology can provide the opportunity to reuse Si-NW biosensors and generally improve their efficiency. However, removing covalent bonds on the surface of nanowires with oxygen plasma or strong oxide can damage the nanowires and reduce the sensitivity of the sensor.

3. Stability over time. Issues such as sensor degradation, drift, or change in sensitivity over time may affect the reliability of the analysis, especially in applications requiring continuous monitoring.

4. It is necessary to neutralize the shielding effect to improve the sensor sensitivity. The potential field generated by the target molecule on the surface can be shielded; this prevents the attraction of carrier changes in the Si-NW channel and ultimately reduces the sensor sensitivity.

5. Miniaturization of portable and wearable devices with maintaining sensor performance can be a challenging task. In addition, the development of suitable protection for Si-NW biosensors from external influences and environmental conditions is critical for their practical use.

6. To be widely adopted, Si-NW biosensors should be user-friendly and accessible to people without special training. Simplification of the user interface, data interpretation, and maintenance procedures is essential for their successful integration into clinical diagnostics.

7. Complexity of equipment registration. Manufacturing methods, especially those requiring specialized equipment, face difficulties in registration and certification; this can slow down their commercial adoption and increase development and production costs.

Despite the current preliminary stage of development and the problems faced by developers of commercial Si-NW biosensors, there is a significant potential for their application, determined by: (i) high sensitivity of the analysis; (ii) short signal recording time, the possibility of multiplex analysis, and miniaturization of the device; (iii) cost-effectiveness of production. Application is possible both in research and clinical practice. Subfemtomolar sensitivity of real-time analysis without the need for amplification and the use of labels, as well as without the use of large amounts of biological material, can potentially significantly simplify and accelerate the process of detecting various diseases at early stages. This, in turn, can increase the effectiveness of treatment and improve the prognosis for patients. In addition, the wide possibilities of Si-NW biosensors for integration into portable and wearable devices open up new prospects for continuous monitoring of health in real time, which can contribute to the development of personalized medicine and effective monitoring of patients' health.

2. APPLICATION EXAMPLES OF Si-NW BIOSENSORS FOR BIOMOLECULE DETECTION

The high sensitivity and usability of Si-NW biosensors make them promising candidates for a wide range of diagnostic and biomedical applications. Table 1 summarizes the research results in the field of medical applications of Si-NW biosensors. These include the development of *in vitro* diagnostic devices, one of the most common applications of Si-NW biosensors. However, most of the research in the field of using Si-NW biosensors in medical diagnostics has focused on the development of integrated platforms compatible with the clinical environment.

The following conclusions can be drawn using the data presented in Table 1:

1. The results of the studies presented in Table 1 cover a wide range of diseases, including cancer (ovarian, lung, breast, liver, prostate, etc.), viral infections (hepatitis B, Dengue fever, SARS-CoV-2), autism, cardiovascular diseases, and others. The range of target molecules includes proteins (e.g., CA 125, PSA), microRNA, circulating tumor DNA, viral biomarkers (e.g., SARS-CoV-2 spike protein), and viral particles.

2. The presented results are characterized by extremely low detection limits, reaching attomolar (aM) concentrations. For example, the detection limit for microRNA-21 in two different studies is 10 aM [47, 71], and for circulating tumor DNA (IK3CA E542K) it is 10 aM [38].

3. The conducted research is aimed at developing biosensors for use in clinical practice or for real-time health monitoring. For example, sensors for detecting SARS-CoV-2 (spike protein and N protein) [41, 72] and glucose in diabetes mellitus [40] are especially needed for practical application.

As can be seen from Table 1, Si-NW biosensors demonstrate high sensitivity, the ability to perform multiplex analysis, and the potential for use in clinical and field practice. Given their high sensitivity and accuracy, Si-NW biosensors can become the basis for new diagnostic devices that can be introduced into medical practice, improving the quality and speed of diagnostics, as well as providing new tools for health monitoring and early detection of diseases.

Table 1. Studies on the medical applications of Si-NW biosensors

Disease	Target molecule/disease marker	Limit of detection	Year	References
Ovarian cancer	Glycoprotein (CA 125)	2.2×10^{-16} M	2020	[10]
Ovarian cancer	Glycoprotein (CA 125)	2×10^{-17} M	2021	[35]
Glioma	Circular RNA (circ-NFIX)	1.1×10^{-17} M	2021	[9]
Glioma	Circular RNA (circ-SHKBP1)	1.1×10^{-16} M	2021	[7]
Glioma	MicroRNA-363	3.3×10^{-17} M	2021	[36]
Colorectal cancer	MicroRNA-17-3p	1.1×10^{-17} M	2021	[11]
Autism	MicroRNA-106a-5p, microRNA-106b-5p, microRNA-494-5p, microRNA-15b-5p	1.1×10^{-17} M	2022	[8]
Ovarian cancer	MicroRNA-21, microRNA-141, microRNA-200a	1.1×10^{-16} M	2022	[37]
Cancer	Circulating tumor DNA (PIK3CA E542K)	10 aM	2021	[38]
Acute kidney injury	Human serum cystatin C (Cys-C)	0.25 ag/ml	2023	[39]
Diabetes mellitus	Glucose	10 nM	2023	[40]
SARS-CoV-2	Spike-protein	100 ng/ml (or 575 pM)	2022	[41]
Neurological disorders	γ-Aminobutyric acid (GABA)	970 fM to 9.7 μM	2019	[42]
Cancer	Serum carcinoembryonic antigen (CEA)	10 fg/ml	2019	[43]
Prostate cancer	Prostate specific antigen (PSA)	23 fg/ml	2017	[44]
Dengue fever	Single-stranded virus DNA	4.131×10^{-13} M	2022	[45]
Hepatitis B	HBV surface antigen (HBsAg) and HBV X protein (HBx)	12 fM and 40 fM	2021	[46]
Cancer	MicroRNA-21	10 aM	2022	[47]
Liver cancer	Carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP)	0.1 fg ml^{-1} (AFP) and 1 fg ml^{-1} (CEA)	2022	[48]
COVID-19	Interleukin-6 (IL-6)	2.1 pg/ml	2023	[49]
Bacterial infections	*Escherichia coli*	10^2 CFU/ml	2022	[50]
Cardiovascular diseases	Cardiac troponin I (cTnI)	0.016 ng/ml	2020	[51]
Breast cancer	Carbohydrate antigens 15-3 (CA15-3) and carcinoma embryonic antigens (CEA)	0.1 U/ml CA15-3 and 0.01 ng/ml CEA	2022	[52]
Tuberculosis	*Mycobacterium tuberculosis* protein	0.01 fg/ml	2021	[53]
Tuberculosis	*Mycobacterium tuberculosis* protein	78.541 fM	2023	[54]
Hepatitis B	Hepatitis B surface antigen (HBsAg)	800 fg/ml	2022	[55]
Cardiovascular diseases	Cardiac troponin I (cTnI)	1.47 pg ml^{-1}	2022	[56]
Lung cancer	MicroRNA-126 and serum carcinoembryonic antigen (CEA)	0.1 fM (microRNA-126) and 1 fg/ml (CEA)	2017	[57]
Hepatitis B	Hepatitis B surface antigen (HBsAg)	2.92×10^{-3} pg/mm^2	2022	[58]
Reproductive system diseases	Follicle-stimulating hormone (FSH)	0.72 fM (buffer) and 1.1 fM (20% serum)	2018	[59]
Influenza	HA1 domain of hemagglutinin (HA)	1 fM	2019	[60]
Prostate cancer	MicroRNA-198 and microRNA-429	3.3×10^{-16} M	2019	[61]
SARS-CoV-2	Omicron	Four effective copies (cps)	2022	[62]
Cardiovascular diseases	Cardiac troponin I (cTnI)	1.36 pg/ml	2023	[63]
Liver failure	Acetaminophen (antipyretic)	From 0.01 mmol to 3 mmol dm^{-3}	2018	[64]
Ovarian cancer	Glycoprotein (CA 125) and human epididymis protein 4 (HE4)	2.5 U/ml CA125 and 3.12 pM HE4	2023	[65]
Ovarian cancer, glioblastoma, colorectal cancer etc.	MicroRNA-21	~10^{-16} M	2018	[66]

Table 1. Studies on the medical applications of Si-NW biosensors (continued)

Disease	Target molecule/disease marker	Limit of detection	Year	References
Cancer, neurodegenerative, and cardiovascular diseases	Small extracellular vesicles (sEVs)	2×10^5 sEVs/ml	2021	[67]
Influenza	Influenza A virions	6×10^{-16} M	2021	[68]
Oxidative stress	Glutathione and malondialdehyde	50 nM and 3.2 nM for aqueous solutions of glutathione and malondialdehyde, respectively	2023	[69]
Severe pathophysiological conditions	Interleukin-4 (IL-4) and Interleukin -2 (IL-2)	3−5 fM	2020	[70]
Cancer	MicroRNA-21	1 aM	2024	[71]
SARS-CoV-2	N-protein	1 ng ml^{-1}	2024	[72]
Hepatitis B	Genome	2 copies/reaction for the synthetic genome and 20 copies/reaction for the genome extracted from human blood	2018	[73]
Nasopharyngeal carcinoma	Epstein-Barr virus DNA	10^{-13} M	2019	[74]

CONCLUSIONS

Si-NW biosensors are promising devices; their characteristics and capabilities make them convenient tools for solving various problems related to the detection and analysis of biological objects. The global biosensor market demonstrates rapid growth, driven by growing demand in various sectors such as healthcare, environmental monitoring, and food safety. This trend is reflected in the expansion of research and development efforts both in Russia and worldwide.

The unique properties of Si-NW, the possibility of multiplex analysis and compatibility with existing semiconductor manufacturing technologies make Si-NW biosensors promising candidates for use in bioanalysis systems requiring high sensitivity and specificity. Many studies using Si-NW biosensors have shown wide possibilities in detecting various biomolecules, including proteins, nucleic acids, and viral particles, with detection limits reaching the femtomolar range and below.

However, despite numerous advantages, commercialization of Si-NW biosensors and their implementation in biomedical practice faces several challenges. One of the main obstacles is the reproducibility and scalability of silicon nanowire fabrication methods, which are critical for successful commercialization. In addition, the integration of Si-NW biosensors into portable and user-friendly devices requires innovations in both material science and engineering. Despite numerous reports about successful detection of biomarkers of various diseases in buffer solution, direct detection in real samples such as whole blood, serum, and biopsy samples remains challenging due to the high ion concentration of biological materials and high background noise arising from non-specific binding of biological fluid components. Solution of these problems will be important for the transition of Si-NW biosensors from laboratory use to their commercialization and widespread use in both medical laboratories and point-of-care settings. Advances in nanofabrication techniques coupled with a better understanding of nanowire-biomolecule interactions will open the way for more reliable and universal biosensor platforms. Additionally, the use of artificial intelligence and machine learning to analyze and interpret data will further enhance the capabilities of these biosensors, potentially enabling more accurate and rapid diagnostics. As research continues to address emerging challenges over time, future commercialization and integration of biosensor technologies into various healthcare applications are quite likely and will have a significant impact on various aspects of human health.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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БИОСЕНСОРЫ НА ОСНОВЕ НАНОПРОВОЛОК ДЛЯ РЕШЕНИЯ БИОМЕДИЦИНСКИХ ЗАДАЧ

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Рассмотрены современные достижения и перспективы использования нанопроволочных биосенсоров. Обсуждаются принципы их действия, методы изготовления и влияние эффекта Дебая, который играет ключевую роль в улучшении их характеристик. Особое внимание уделено практическому применению таких биосенсоров для детекции разнообразных биомолекул, демонстрирующему их возможности и потенциал в обнаружении широкого спектра биомаркеров различных заболеваний. Благодаря своей высокой чувствительности и специфичности, они также показывают превосходные результаты в таких областях, как ранняя диагностика заболеваний, мониторинг состояния здоровья пациентов и персонализированная медицина. Учитывая их высокую эффективность и многообразие приложений, биосенсоры на основе нанопроволок обладают значительными перспективами для коммерциализации и широкого применения в медицине и смежных областях, что делает их важным направлением для будущих исследований и разработок.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: биосенсор; кремний; нанопроволоки; биомедицина; диагностика

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