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Biosensors: Design, Classification and Application

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The data of modern scientific research on the structure of biosensors, their classification and use in various fields of human practice are presented. Varieties of biosensors are characterized based on the type of bioreceptor and physical-chemical transducer in their structure. The peculiarities of the structure of biosensors on the basis of enzymes, cells, cellular organelles, tissues, nucleic acids, antibodies, and aptamers are reviewed. The principles of functioning of optical, acoustic, calorimetric, piezoelectric, and electrochemical biosensors are described. The data on the design of a new generation of biosensors are summarized. The effectiveness of various methods of bioreceptor immobilization is compared. Physical (physical adsorption, incorporation into the matrix, encapsulation) and chemical (covalent binding, cross-molecular interactions) methods of bioreceptor immobilization are described. Methods of improvement of electrochemical properties of biosensors by including carbon nanomaterials (nanotubes, graphene, graphene oxide) and metal nanoparticles in their composition are considered. Examples of the use of biosensors for assessing the quality of food and drinking water, monitoring technological processes in various industries, determining the level of environmental pollution with toxic compounds, monitoring of human health indicators, identifying micro-organisms and their toxins that can be used as biological weapons, etc. are provided. Further prospects for the development of biosensor technologies are discussed.

Keywords: Biosensor, Nanomaterials, Bioreceptor, Physico-chemical transducer.

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1. INTRODUCTION

The development and implementation of sensor with different functions [1, 2] in practical use are one of the priority areas of modern electronics.

Development of biosensors requires integration of knowledge in molecular biology, enzymology, genetic and protein engineering, analytical chemistry, materials science, microelectronics, and nanotechnology [3]. The global biosensor market is growing rapidly: in 1983, it was less than 5 million US dollars a year [4], and in 2018 it exceeded 18.9 billion [5]. The biosensor market is very competitive and is mainly driven by the medical and pharmaceutical sector. Biosensors are analytical devices that use biochemical reactions to detect a wide range of chemical compounds: metabolites, medications, environmental pollutants, etc. [6-8].

The first biosensors were created by the American biochemist Leland Clark: in 1956, the scientist developed a biosensor for oxygen content measurement, and in 1962 – glucose concentration measurement. Today, glucose meters are the most common biosensors; they account for more than 90 % of the global biosensor market [9]. In addition to health care, biosensors are used in environmental protection, pharmaceutical and biotechnological production, food processing, agriculture, as well as national security and defense [10, 11]. Biosensors are tools for scientific research. Biosensors are used to study gene expression at the cellular level, localization, and dynamics of enzyme substrates, receptors, reactive oxygen species, etc. [12].

Biosensors are an alternative for analytical research methods that are technologically complex and require expensive equipment and significant time expenditure.

The advantages of biosensor use are the following: high sensitivity and accuracy of measurements – biosensors are able to detect substances in the amount of 10-12 g or less; selectivity; determination of substances does not require preliminary preparation of an analytical sample; the ability of continuous monitoring; speed and ease of measurement; safety in use; low cost.

The aim of this study is to analyze and generalize the results of modern scientific research on the functional features of new-generation biosensors, their structure, classification, and scope of practical application.

2. STRUCTURE AND CLASSIFICATION OF BIOSENSORS

Biosensors are analytical devices, the sensory systems of which have biochemical nature and are based on reactions involving biomolecules or supramolecular structures. The biosensors consist of three components: bioreceptor – detector layer of immobilized biomaterial; physico-chemical transducer, which is able to transform the biological response into a measured signal; an electronic system for amplifying and recording the signal (see Fig. 1) [13].

There are two categories of bioreceptors: catalytic and affine. Catalytic receptors catalyze the biochemical reaction, while affine ones provide binding of specific molecules. The catalytic bioreceptors include enzymes, microbial cells, and tissues. Biosensors based on catalytic bioreceptors allow performing analysis in a continuous mode to determine substances in concentrations from micro- to millimoles. The disadvantage of these sensors is low stability. Affine bioreceptors include antibodies, cell receptors, nucleic acids, and aptamers. Biosensors based

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on affine receptors are designed for single determination of hormones, steroids, and medications in concentrations from pico- to micromoles [8].

Depending on the type of bioreceptor, biosensors are classified into enzymatic, cellular, tissular, DNA sensors, immunosensors, and aptasensors. Biosensors are also classified by the type of transducer, which can be optical, calorimetric, acoustic, piezoelectric, and electrochemical.

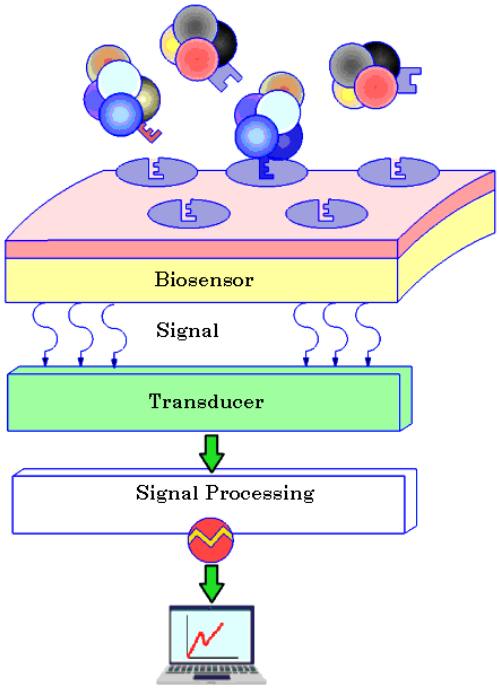

Fig. 1 – Diagram of the structure and operating principle of biosensors

2.1 Enzymatic Biosensors

Enzymatic biosensors are divided into substrate and inhibitor ones. Substrate biosensors determine the concentration of substrates of enzymatic reactions. Michaelis-Menten equation is used for quantitative determination of the concentration of the analyzed substrates in enzymatic biosensors:

$$\nu = \frac{\nu_{\text{m}} \cdot S}{S + K_m},$$

where ν is the initial velocity, ν_{m} is the maximum velocity achieved by the system at saturating substrate concentrations, S is the substrate concentration, K_m is the substrate concentration at half of the ν_{m}.

For example, biosensors on the basis of glucose oxidase determine the concentration of glucose, on the basis of urease – urea. Inhibitor sensors are designed to determine the concentration of substances that reduce the enzyme activity. For example, biosensors based on acetylcholinesterase determine the concentration of organophosphate pesticides, which are enzyme inhibitors.

2.2 Cellular Biosensors

Cellular biosensors are used to study complex metabolic processes, such as biochemical oxygen consumption, photosynthesis, and mutagenesis. Bacterial, fungal, yeast, algae, protozoa cells and cell cultures are used as bioreceptors of these sensors. Biosensors based on microbial cells are called respiratory sensors, because during the absorption of organic substances, they change the amount of biochemical oxygen consumption. This characteristic of microbial biosensors determines their use for detecting a wide range of pollutants: heavy metals, pesticides, herbicides, etc. [14, 15].

The principle of operation of a typical cell-based biosensor is the detection of a specific type of analyte and amplification of this identification into an electrical and optical signal via processor. Since the analyte enters the bacterial cell by diffusion, the rate of diffusion of the analyte is the cause of a delayed sensor response. The effectiveness of cellular biosensors for detecting environmental pollutants depends on the reporter genes in the cell genome. A reporter is a gene that is adapted by genetic engineering to another gene under study, or to cell culture, so that the expression or localization of the product of this gene in the cell can be visualized. Most common reporters are the luciferase gene (Luc) or the green fluorescent protein gene. Using genetic constructs (plasmids) that contain reporter genes, it is possible to study transcriptional activity in cells.

Depending on the method of the reporter gene expression, biosensors on the basis of bacterial cells can be classified into two types: constitutive and inducible. In constitutive type systems, the reporter is expressed in the bacterial cell at a high basal level. An increase in the amount of cell-toxic substances causes its death, thereby leading to a decrease in the amount of the synthesized reporter protein, and, consequently, to a decrease in bioluminescence and a reduction in signal generation. In biosensors with an inducible gene expression system, cells constructed by genetic engineering methods, contain a plasmid in which an inducible promoter, associated with a reporter gene, controls its expression. In the absence of an analyte/inducer, the reporter gene is expressed at a very low basal level. In contrast, in the presence of an analyte/inducer, it is expressed in a dose-dependent manner. Biosensors, constructed using induced promoters, are characterized by both specificity and high sensitivity, which is determined by the interaction of a regulatory protein (transcription repressor or activator) with a chemical substance.

Inducible biosensors can be further classified into stress-inducible and chemically-inducible, depending on the response activation mechanism. In stress-inducible cellular biosensors, the reporter gene is controlled by a promoter that is activated under stressful conditions, such as during heat shock and osmotic stress. Biosensors of this type are semi-specific.

On the other hand, a biosensor with a chemically-inducible gene expression system contains a specific promoter and genes for regulatory and reporter proteins. The presence of the analyte activates the promoter in chemically-inducible bacterial biosensors, which in turn triggers the expression of the regulator and reporter gene in a certain way. The mechanism by which this occurs involves binding of the analyte to a regulatory protein that undergoes conformational changes and subsequently activates the reporter gene expression.

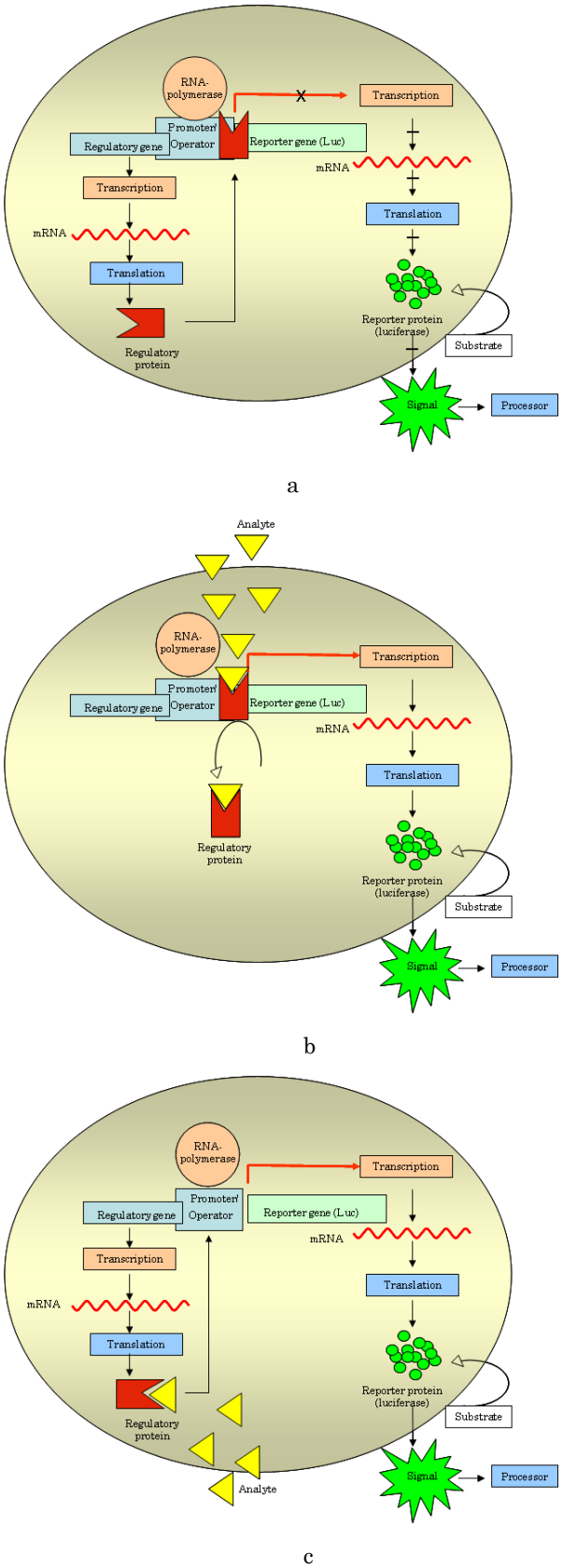

Fig. 2 – Inducible cellular biosensors: negative regulation of the reporter gene expression without the participation of the analyte (a), in the presence of the analyte (b), positive regulation of the reporter gene expression (c)

The reporter gene expression may be under positive or negative regulation. In the case of negative regulation (Fig. 2a), the regulatory protein binds to the operator/ promoter site of the operon and blocks transcription of structural genes, including the reporter gene, by RNA polymerase. In the presence of the analyte, the latter binds to a regulatory protein, which in turn separates from the operator/promoter regions of the operon, triggering the reporter gene expression (Fig. 2b). In case of positive regulation (Fig. 2c), the analyte firstly binds to the regulatory protein, and the formed complex subsequently binds to the operator/promoter, causing the reporter gene expression [16].

Thus, cell-based biosensors are able to measure the effect of the analyte under study on cellular metabolism, namely: secondary messengers and intracellular enzymes; detect toxic effects and side effects of the studied substances on cells; inhibiting or stimulating effect on the studied receptor, etc. The example could be a plasmid, in which a specific regulatory sequence was placed before the luciferase gene, which affects the expression of a bioluminescent protein in the presence of the analyte. Due to this design, it is possible to analyze substances that disrupt the endocrine system functioning (chlorinated biphenols and various pesticides) by studying their estrogenic and androgenic activity. Reporter genes provide the transformation of a biological response into a signal that can be detected by physical and chemical methods using a transducer. The study authors [5] report the creation of a biosensor based on human hepatoma cells with a green fluorescent protein reporter gene. This sensor is used for quick and easy detection of genotoxic agents. Biosensors based on eucaryotic cell cultures are used to study the biochemical mechanisms of metabolic pathologies and disorders at the cellular level.

Cellular biosensors possess a number of advantages over enzymatic ones: availability, low cost and ease of obtaining bioreceptors, no need for coenzymes, the integrality of the cellular response, long service life. The main disadvantages of cellular biosensors are low selectivity of the sensory response, which is explained by the presence of many enzymes in the cell; loss of mediators via diffusion; rapid cell death.

2.3 Tissue-based Biosensors

Plant and animal tissue materials are used as biocatalytic components of biosensors due to their ability to create a natural environment for the corresponding enzyme. For the most part, tissular biosensors have longer service life compared to enzymatic biosensors. Tissue materials retain a high specific activity compared to enzymes, isolated under the same conditions. Due to many enzymatic processes that occur in cells, special attention should be paid to sensor selectivity. Thus, exposure to a large number of metabolites present in complex biological objects may interfere with the sensor operation. For example, interference occurs as a result of enzymatic reactions, in which a directly detected by an electrochemical sensor product is formed from other substrates, which leads to a change in the *pH* on the electrode surface. To

prevent contamination of the tissue layer with bacteria, an antimicrobial agent (for example, sodium azide) should be introduced into the system. Tissular biosensor optimization procedure involves an experimental study of various sensor parameters: method of tissue layer immobilization, *pH* influence, the concentration of activators and inhibitors, biosensor selectivity. Tissue-based biosensors most often record changes in *pH* and gas concentrations (oxygen, ammonia, carbon dioxide) [6]. The *pH* value of the solution affects both the activity of enzymes and the potential of the gas-sensitive membrane electrode. Suitable activators are introduced into the system to enhance the desired enzymatic reactions and inhibitors to inhibit the activity of enzymes that catalyze adverse reactions. At the same time, it is necessary to make sure that the introduction of an inhibitor does not adversely affect the main enzymatic reaction.

Tissue-based biosensors possess a number of advantages over enzymatic biosensors: enzymes in tissues are in their natural state, so they are less exposed to inactivation; stability and high biocatalytic activity (tissues may work in cases when purified enzymes do not work); lower cost compared to purified enzymes.

Biosensors based on plant tissues (tomatoes, potatoes, horseradish) have been developed to determine the concentration of flavonoids, catechol, dopamine, etc.

2.4 Biosensors Based on Cellular Organelles and Immunosensors

If it is necessary to increase the amount of immobilized enzyme or improve the selectivity of the sensor, eliminating interfering enzymes, biosensors based on subcellular fractions should be used. Some cellular organelles can be used as analytical reagents. Biosensors based on cellular organelles (mitochondria, chloroplasts, liposomes, microsomes) are used as phytotoxicity sensors in the study of the mechanisms of pollutants' action [8].

Immunosensors contain monoclonal antibodies as bioreceptors. Immunoaffinity reaction in immunosensor involves the irreversible binding of the antigen with the antibody. As to form a stable antigen-antibody complex, a high degree of complementarity is required between the epitope (part of the antigen macromolecule) and the paratope (part of the antibody that recognizes the epitope). Most antibodies, used in immunosensors for biomarker (antigen) detection and assay, are monoclonal antibodies and recognize the specific structural sequence of the biomarker. Monoclonal antibody preparations that are specific to a single antigen determinant (epitope) are more specific for the corresponding biomarker, i.e., the antigen than polyclonal antibodies. In immunosensors, a biochemical reaction that leads to the formation of an antigen-antibody complex eventually produces a signal that can be detected using a transducer. In developing biosensors, special attention should be paid to the conditions of the interaction of antibodies with nanomaterials, since different molecules of antibodies possess excellent electrostatic properties, so it is necessary to make appropriate adjustments when creating an antibody-nanomaterial conjugate for different antibodies, even when the same nanomaterials are used. In designing immunosensors, as well as for many enzyme immunoassays, horseradish peroxidase (HRP) or alkaline phosphatase (ALP) enzymes are used as biochemical "labels" for signal registration. In this case, the reacting antibody forms a conjugate with HRP or ALP. Generated electrochemical signal depends on the concentration of the electroactive product, formed as a result of an enzymatic reaction, which is affected by the amount of the "label"-enzyme associated with the immunosensor. The amount of antigen bound by the antibody in the immunosensor, and ultimately the concentration of the antigen in the biological sample, are related to the amount of the enzyme ("label") detected at the detection stage. The use of "labels" provides a significant signal amplification in the immunosensor, since each enzyme molecule quickly generates many product molecules, which are detected electrochemically. An example of immunosensor use with "ALP" enzyme as a "label" is an immunosensor for cortisol detection in saliva samples [17].

Immunosensors possess high specificity, sensitivity, and selectivity. They detect substances in ultra-low concentrations that cannot be measured by traditional chemical analysis methods. Immunosensors are used for the diagnosis of both infectious diseases, such as AIDS, and non-communicable diseases, for example, early diagnosis of breast cancer [16]. Immunosensors have been developed to detect hormones (thyroxine, insulin, chorionic gonadotropin), cancer markers (alphafeto-protein), toxins in food products and the environment (mycotoxins, pesticides, herbicides), pathogenic bacteria and viruses [17, 18].

2.5 DNA-biosensors and Aptasensors

DNA-biosensors are called genosensors. The principle of genosensor operation is based on a specific process of a nucleic acid sample hybridization with a complementary probe. Hybridization probes are usually marked with fluorescent, electrochemical, or radionuclide labels, and are most often used in clinical diagnostics. Diagnostic biochips that allow the analysis of several thousand genes simultaneously have been developed.

Aptasensors contain synthetic bioreceptors – aptamers. The term aptasensor first appeared in 2004 [6]. Aptamers are produced by chemical synthesis and can be easily modified using certain functional groups and linkers [9]. There are two classes of aptamers: nucleic and peptide aptamers. DNA and RNA aptamers consist of 20-80 nucleotides. Aptamers are ultra-selective elements of biological recognition. They are used for specific binding to nucleic acids, proteins, amino-acids, metal ions, dyes, and toxins [6]. Developed biosensors that contain both the aptamer and antibody are used in clinical diagnosis to determine the concentration of thrombin, lysozyme, etc.

In addition to aptamers, polymers with specific binding centers that are complementary in structure and physico-chemical properties to specific molecules are used as synthetic bioreceptors. High affinity and stability of synthetic bioreceptors determine the prospects for their use in the development of new biosensor technologies.

2.6 Optical Biosensors

Bioreceptor's biological response, generated when it interacts with the test substance, is converted into a measured signal using a physico-chemical transducer. Optical biosensors record the changes of the optical properties: absorption, scattering, polarization and refraction of light, fluorescence, luminescence, bioluminescence. The main elements of a fiber-optic sensor are an optical fiber, a light source and a light-receiving device, an optical sensitive element, and special communication lines between these elements. The principle of operation of most optical biosensors is based on the phenomenon of surface plasmon resonance. Optical biosensors to measure *pH*, O_2, CO_2, NH_4^+, NADH, glucose, amino acid, toxin, heavy metal ion concentration, and record changes in luminescence intensity in photobacteria have been developed [3].

Fiber-optic sensors are widely used in medicine, for example, to measure blood *pH*, blood saturation with oxygen and carbon dioxide. Such sensors can be used to measure a fluorescent dye (for example, indocyanine green) concentration, injected into the blood for diagnostic purposes: to assess liver function, determine cardiac output, for ophthalmological angiography, visualization of blood flow in the liver and stomach, diagnosis of liver metastases and cancer, etc. The use of biosensors based on fluorescent proteins allows visualization of biological processes in situ, to study the mechanisms of action of anticancer agents, to detect ions, metabolites, and disease biomarkers [6].

2.7 Piezoelectric Biosensors

Piezoelectric biosensors measure changes in mass that result from the interaction of biomolecules. A change in the mass of the material on the quartz crystal surface causes a quantitative change in its resonant frequency. Piezoelectric immunosensors are biosensors that contain an antibody as a biorecognition element and the specificity of the antibody significantly influences the specificity of the entire immunosensor. Usually, immunosensors contain immobilized antibodies and they are able to recognize the corresponding antigens. However, the opposite reaction is also possible: the immunosensor may contain an immobilized antigen and be used to recognize an antibody, which in this case is the molecule being analyzed. This is why piezoelectric immunosensors with immobilized antigen are a suitable tool for the diagnosis of infectious diseases. Piezoelectric immunosensors are suitable for analytes with high molecular weight, because they cause a higher reduction in the oscillation frequency. However, a limitation for the application of this method is the recognition of low-molecular analytes by antibodies immobilized on a piezoelectric platform. Micro-organisms are a specific group of analytes that can be directly analyzed by piezoelectric immunosensors. Piezoelectric immunosensors are the most sensitive sensors, since they are able to detect antigens in the picogram range.

2.8 Electrochemical Biosensors

Electrochemical biosensors make up 80 % of the total number of biosensors. Electrochemical biosensors are divided into conductometric, potentiometric, and amperometric.

Conductometric biosensors measure the conductivity of electrolyte solutions, which depends on the concentration and mobility of ions. Changes in the conductivity of solutions occur due to the generation of charged products of a biochemical reaction.

Potentiometric biosensors are used for the quantitative determination of substances based on the difference in electrical potentials between the detector electrode that contacts with the analyzed sample and the reference electrode. Ion-selective or gas-sensitive electrodes and field-effect transistors are used as part of potentiometric biosensors. The potential of the indicator electrode is determined by the Nernst equation:

$$E = E^0 + \frac{2.3 \cdot R \cdot T}{z \cdot F} \cdot \lg \frac{[Ox]}{[\mathrm{Re}\, d]},$$

where E is the measured reduction potential under specific conditions, E^0 is the reduction potential at standard-state conditions, R is the ideal gas constant, T is the absolute temperature in K, z is the number of electrons transferred in the balanced equation, F is Faraday's constant (96500 C mol^{-1}), [Ox] and [Red] are the molar concentrations (chemical activities) of substances involved in the corresponding half-reaction in oxidized (Ox) and reduced (Red) forms.

Amperometric biosensors register changes in current density or strength. There are three types of amperometric biosensors: mediator-free, that measure concentrations of electroactive substrates or products of enzymatic reactions; mediator biosensors, in which mediators transfer electrons from the active center of the enzyme to the electrode; biosensors that function on the basis of direct electron transfer between the active center of the enzyme and the electrode [19]. Such biosensors possess high selectivity and sensitivity.

Biosensors based on electrochemical and optical physico-chemical transducers are most often used for commercial purposes to determine the concentration of metabolites and identify infectious agents.

3. DESIGN OF BIOSENSORS

The most crucial step of biosensor design is the immobilization of bioreceptors on the surface of the sensor material which can be metal, glass, polymer, or paper.

There are physical (adsorption, incorporation into the matrix, encapsulation) and chemical (covalent binding, cross-molecular interactions) methods of bioreceptor immobilization (see Fig. 3). The choice of the immobilization method depends on the nature of the bioreceptor, the type of physical and chemical transducer, and the properties of the substance under study [6, 7].

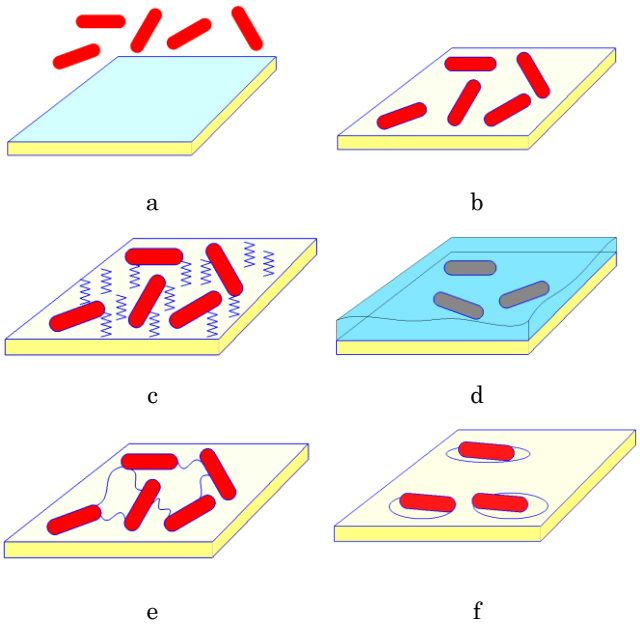

Fig. 3 – Methods used for bioreceptor immobilization in biosensors: before immobilization (a), adsorption (b), covalent binding (c), matrix entrapment (d), cross-linking (e), encapsulation (f)

3.1 Physical Methods of Bioreceptor Immobilization

Physical methods of bioreceptor immobilization are used in the creation of enzymatic and cellular biosensors. Physical adsorption on an insoluble carrier is the easiest way to immobilize enzymes, which does not cause their denaturation. Physical adsorption is based on the combined action of van der Waals, hydrophobic, ionic, and hydrogen interactions that cause the bioreceptor to attach to the sensor surface. The advantage of this method is its simplicity and low cost; the disadvantage is the low stability of the formed complexes [15]. Reagent washout is particularly significant when the reagent molecules are smaller than the matrix pores, and there is no additional binding to the matrix. In this case, immobilization should combine physical and chemical methods.

When immobilizing bioreceptors by incorporating them into the matrix, gel matrices based on polyacrylamide, starch, polyvinyl alcohol, polyvinyl chloride, polycarbonates, cellulose acetate, alginate, and silica gel are most often used [22].

Bioreceptors are encapsulated in a porous matrix using the "sol-gel" system. "Sol-gel" processes occur at room temperature, which prevents denaturation of biomolecules, and the resulting structures are characterized by high stability. Proteins, enzymes and antibodies have been successfully encapsulated in "sol-gels" for biosensors applications. During the encapsulation of bioreceptors, a polymer porous matrix is formed around them, which can be obtained by gelation from a solution of gelatin, agar, alginate, polyacrylamide, or by polycondensation of some organic esters or acid chlorides.

Physical adsorption is the simplest type of immobilization, which does not always meet the requirements for designing biosensors, because if the surface itself is not changed or the activity of the adsorbed particles is not affected, it is almost impossible to control the attachment sites.

3.2 Chemical Methods of Bioreceptor Immobilization

Chemical methods of immobilization involve the formation of additional chemical bonds between the functional groups of bioreceptor molecules and specific reagents. The covalent binding provides modification of the sensor surface by specific reagents containing active functional groups that can bind to bioreceptor molecules. Following nucleophilic groups participate in the formation of covalent bonds as part of enzymes: thiol, hydroxyl, carboxyl, and amino groups. Due to covalent binding, bioreceptors are evenly distributed on the sensor surface.

In addition to the fact that one of the reagents is stationary, and the behavior of the other is controlled by diffusion, there is the main difference between the immobilization process and the bimolecular reaction in a homogeneous phase. In the homogeneous phase, the reactivity of reagents remains constant regardless of the reaction rate, until one of the components begins to limit it. In the process of immobilization, individual adsorption centers can not be considered independent of each other; adsorption on one of the centers can significantly affect the reactivity of neighboring ones; thus, the reaction kinetics changes over time.

Chemical binding is characterized by large adsorption enthalpy, high orientational selectivity and stability of the layers compared to the physical binding. After the biosensor surface formation, its interaction with the analyte will not be homogeneous, and will also include chemosorption.

During immobilization of bioreceptors by cross-intermolecular interactions, bifunctional and polyfunctional reagents, such as glutaric aldehyde, hexamethylene diisocyanate, 1.5-difluoro-2.4-dinitrobenzene are used. The disadvantage of this immobilization method is the inhomogeneity of the formed surface, which may contain several receptor layers. The method of cross-intermolecular interactions is most often used for the immobilization of aptamers and proteins.

4. NANOMATERIALS AS BIOSENSOR COMPONENTS

Nanotechnologies play a crucial role in the development of modern biosensor technologies. The use of nanomaterials in biosensors improves mechanical, optical, magnetic and electrochemical characteristics of these devices [7]. Nanomaterials can be immobilized on the surface of the transducer as part of a bioreceptor component, or the transducer can be modified by a nanomaterial. Nanomaterials have a high level of surface area/volume ratio, are characterized by high electrical conductivity, catalytic activity, adsorption capacity, etc. Due to the presence of functional groups (–COOH, $–NH_2$, –SH) in the enzyme structure, they can be easily immobilized on nanoparticles. As adsorbents for enzymes, inorganic mesoporous materials with a

large surface area of the crystal and capable of carrying a variety of chemical groups can be used.

Electrochemical biosensors based on carbon nanomaterials have been developed to determine the glucose, dopamine, serotonin, ascorbic acid, acetylcholine, amino acid, lactate, cholesterol, protein, enzyme, uric acid, cortisol, glutamic acid, hydrogen peroxide, and nucleic acid content. Carbon nanomaterials in biosensors increase the electroactive surface area, enhance electron transport, and promote adsorption of molecules. Modification of the sensor surface with carbon nanomaterials (nanotubes, graphene) improves the immobilization of enzymes and cells. It was found that 9600 enzyme molecules can be attached to a carbon nanotube of 1 μm in length [6]. The combination of several nanomaterials in electrode surface modification provides the formation of nanocomposites that improve stability and increase the sensitivity of biosensors. Biosensors have been developed for the determination of glucose, xanthine and hypoxanthine concentration, in which electrode surface is modified with carbon nanotubes in combination with gold nanoparticles [19].

The inclusion of metal nanoparticles (Au, Ag, Co, Cu, Pt, Fe, Ni, Pd) and their oxides (TiO_2, ZnO) in biosensors increases sensitivity and specificity of electrochemical catalysis. Gold nanoparticles possess high affinity for antibodies, so they are often included in immunosensors. Gold nanoparticle conjugates with specific antibodies to cancer cell receptors are used in the diagnosis of oncological diseases [6]. The ability to form strong thiol bonds between organic substances and the surface of gold nanoparticles is used in the development of biosensors on the basis of enzymes. In particular, potentiometric biosensor on the basis of urease bionanoconjugate with gold nanoparticles was developed for the detection of glyphosate pesticide. Such a biosensor's operation principle is in inhibition of urease activity by glyphosate, which led to a decrease in the response of the ammonium-sensitive ion-selective electrode. An electrochemical biosensor on the basis of glucose oxidase, immobilized together with a nanocomposite on the basis of platinum nanoparticles and SnS_2 is described. According to the authors, this biosensor demonstrates excellent selectivity and is successfully used for the determination of glucose in human serum samples. Glucose oxidase, immobilized on a nanocomposite, demonstrates good bioactivity. The use of nanocomposite improved direct electron transfer between glucose oxidase and the surface of the glassy carbon electrode.

A photoelectrochemical biosensor on the basis of glucose dehydrogenase, immobilized on an electrode covered with quantum dots (ZnS-CdS) and multilayer carbon nanotubes, is proposed. The biosensor is used to determine glucose in amperometric and photo-amperometric modes, and nanomaterials interacted in the enzymatic reaction with NADH formation and generated the biosensor response. To determine the concentration of total cholesterol, an electrochemical biosensor on the basis of two-enzyme cholesterol esterase/cholesterol oxidase, immobilized into a composite matrix made of quantum dots (CdS) and chitosan, was developed. The use of quantum dots in biosensors makes it possible to create a new generation of devices used for malignant tumor detection and visualization, diagnosis of heart attacks and strokes, viral infections [10].

5. APPLICATION OF BIOSENSORS

The main application areas of biosensors are food industry, medicine, environmental monitoring and protection (see Table 1).

In the environmental protection area, biosensors are used to assess chemical (heavy metal salts, phenolic compounds, pesticides, herbicides, insecticides) and biological (toxins, antibiotics, and micro-organisms) environmental pollution. Urease-based conductometric biosensors are used to determine the concentration of heavy metal ions (Hg, Cu, Cd, Co, Pb, Sr etc.).

In the food industry, biosensors are used to determine the composition of food products and control fermentation processes. Biosensors for detection of glycerol, phenols, organic acids (lactic, malic, acetic), and vitamin C have been developed [5, 7].

Aptasensors are used to determine GMO. The use of biosensors can significantly reduce the analysis time and increase its sensitivity. Traditional microbiological methods for the analysis of food contamination by pathogens require a few days, while this analysis using biosensors is performed in less than a day. Cellular and enzymatic multibiosensors that allow simultaneous detection of different pollutants have been developed. For example, a biosensor on the basis of a three-enzyme Biosensors on the basis of *Arthrobacter globiformis* and *Photobacterium phosphoreum* bacteria cells are used to assess industrial wastewater pollution [20]. Bacterial sensors determine biochemical oxygen consumption by microbial cells, which reflects the content of pollutants in water [14]. Biosensors, whose operation principle is based on the inhibition of acetylcholinesterase and cholinoxidase enzymes, are used to detect pesticides [9].

However, such sensors are not selective, since acetylcholinesterase is inhibited by several substances. Aptasensors that detect numerous pollutants, such as heavy metal ions (Hg^{2+}, Pb^{2+}), pesticides (methyl-parathion, methylparaoxone, chlorpyrifos), insecticides (acetamiprid), fungicides (carbendazim), etc., possess high selectivity of action [6, 15].

In medicine, biosensors are used to determine biochemical components of human blood (glucose, urea, cholesterol, etc.), identify disease biomarkers, and molecular diagnostics of inherited diseases [6, 10].

Biosensors make up more than 80 % of devices used in clinical diagnostics. The use of biosensors allows detecting oncological, immunological, neurodegenerative, cardiovascular [7] and infectious diseases at early stages [6, 12]. Improvement of early disease diagnosis contributes to the transition from therapeutic to preventive medicine.

Table 1 – Application of biosensors on the basis of enzymes and microbial cells

Application areas	Determined substance	Enzymes or cells of microorganisms
Food industry	Starch	*α*-Amylase
	Maltose	*α*-Glucosidase
	Caffeine	*Pseudomonas Alcaligenes*
	Glutamine	*Sarcina flava*
	Fructose	Fructose dehydrogenase
	Lactic acid	Lactate oxidase
	Sucrose	Mutarotase *Saccaramicies cerevisiae*
	Ethanol	*Acetobacter aceti*
	Asparagine	Asparaginase
	Lysine	*Escherichia coli*
Environmental monitoring	Herbicides	Tyrosinase *Ostreococcus tauri* *Cictyosphaerium chlorelloids* *Desmodesmus subspicatus* *Scenedesmus intermedius*
	Pesticides	Acetylcholinesterase Carboxylesterase Aldehyde dehydrogenase Glutamate dehydrogenase Urease
	Heavy metal ions: Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Cr^{3+}	Urease *Pseudomonas putida* *Clorella vulgaris*
	Nitrates	*Azotobacter vinelaudi*
	Selenium	Carboxylesterase
	Phenol	Tyrosinase *Trichosporon cutaneum*
	Formaldehyde	Formaldehyde dehydrogenase Alcohol oxidase *Hansenula polymorpha* *Saccaramicies cerevisiae* *Candida maltosa*
Medicine	Glucose	Glucose oxidase *Saccaramicies cerevisiae*
	Cholesterol	Cholesterol oxidase
	Urea	Urease
	Ascorbic acid	*Enterobacter agglomerans*
	Creatinine	Creatinase
	Xanthine, hypoxanthine	Xanthine oxidase
	Pyruvate	*Streptococcus faecium*
	H_2O_2	Peroxidase
	Nicotinic acid	*Lactobacillus arabinosa*
	Uric Acid	*Pichia membranaefaciens*
	Acetylcholine	Acetylcholinesterase
	Dopamine	Tyrosinase
	NAD^+	*Escherichia coli*
	Penicillin	*β*-Lactamase
	Cephalosporin	*Citrobacter freundii*

Implantable biosensors that detect changes in the concentration of metabolites and release medications into the bloodstream using an infusion pump have been developed. An example of such a biosensor is an artificial pancreas, implanted in the abdominal cavity and regulating glucose concentration in the blood of patients with insulin-dependent diabetes mellitus for 7 days [4].

Technologies for the manufacture of disposable biosensors using screen printing are rapidly developing. Modern printing capabilities allow creating almost any biosensor components, which will significantly reduce their cost and facilitate their mass production. Electrochemical printed biosensors provide the ability to monitor health indicators at home [4, 21]. The main requirements for the development of biosensors for "on-site" analysis are their portability, ease of use, and sufficient informativity. Biosensors, integrated directly into the skin (in the form of tattoo) that can be used for non-invasive determination of key electrolytes and metabolites in real time, in particular for determination

of lactate, ammonium and calcium concentration in the sweat of athletes, have been developed. A combination of biosensors with smartphones for detecting various substances and diagnostics is also proposed.

In the biosecurity and defense area, biosensors are used to detect bacteria, viruses and their toxins that can be used as biological weapons. Biosensors are used to detect such toxic compounds as chemical warfare agents with neuroparalytic action (soman). Thus, biosensors play an essential role in ensuring biosafety control and countering bioterrorism.

6. CONCLUSIONS

Biosensors are portable analytical devices that allow performing real-time measurements. High sensitivity, selectivity, accuracy, speed and simplicity of measurements using biosensors determine a wide range of areas of practical application. The future development potential of biosensor technologies is associated with the creation of nanobiosensors for medical diagnostics and eco-monitoring. Nanomaterials, such as quantum dots, magnetic nanoparticles, etc., will become an integral part of biosensors. Further development of biosensors based on 3D printed materials is expected.

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Біосенсори: будова, класифікація та застосування

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Узагальнено дані сучасних наукових досліджень про будову біосенсорів, їх класифікацію та використання у різних галузях практичної діяльності людини. Охарактеризовано різновиди біосенсорів залежно від типу біорецептора і фізико-хімічного перетворювача у їх складі. Розглянуто особливості будови біосенсорів на основі ферментів, клітин, клітинних органел, тканин, нуклеїнових кислот, антитіл, аптамерів. Описано принципи функціонування оптичних, акустичних, калориметричних, п'єзоелектричних, електрохімічних біосенсорів. Узагальнено дані про конструювання біосенсорів нового покоління. Проведено порівняння ефективності різних методів іммобілізації біорецепторів. Охарактеризовано фізичні (фізична адсорбція, включення у матрицю, інкапсулювання) та хімічні (ковалентне зв'язування, перехресні міжмолекулярні взаємодії) методи іммобілізації біорецепторів. Розглянуто способи покращення електрохімічних властивостей біосенсорів шляхом включення до їх складу вуглецевих наноматеріалів (нанотрубки, графен, оксид графену) та наночастинок металів. Наведено приклади застосування біосенсорів для оцінки якості харчових продуктів та питної води, контролю технологічних процесів у різних галузях промисловості, визначення рівня забруднення довкілля токсичними сполуками, моніторингу показників стану здоров'я людини, виявлення мікроорганізмів та їх токсинів, що можуть бути використані в якості біологічної зброї та ін. Обговорюються подальші перспективи розвитку біосенсорних технологій.

Ключові слова: Біосенсор, Наноматеріали, Біорецептор, Фізико-хімічний перетворювач.