Int. J. Electrochem. Sci., 12 **(2017)** 7386 – 7403, doi: 10.20964/2017.08.02

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Review

Main streams in the Construction of Biosensors and Their Applications

Pavla Martinkova[1], *Adam Kostelnik*[1,2], *Tomas Valek*[1], *Miroslav Pohanka*[1,3]

[1] Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 50001 Hradec Kralove, Czech Republic
[2] Faculty of Chemical Technology, University of Pardubice, Studentska 95, 53210 Pardubice, Czech Republic
[3] Department of Geology and Pedology, Mendel University in Brno, Czech Republic
[*]E-mail: miroslav.pohanka@gmail.com

Received: 29 March 2017 / *Accepted:* 22 May 2017 / *Published:* 12 July 2017

Biosensors are quite simple but accurate analytical devices which have been practically used since the half of 20th century and their popularity is still growing. This review is focused on description and summarization of general biosensors construction with introducing of practical examples including graphical explanations. The most applied biorecognition elements like enzymes, genetic information, antibodies, viable cells and, types of standard immobilization techniques and usually used transducers like the optical and electrochemical are given here. Current top topics in biosensing, modern approaches, novel materials and principals and unique combination of transducers and biorecogniction elements are also explained. Survey of actual literature is also provided.

Keywords: Biosensor; biorecognition element; transducer; immobilization method; nanotechnologies

1. INTRODUCTION

Biosensors are characterized as simple measuring devices with a fast response suitable for measurement of wide spectrum of biological or chemical markers in different practical applications (biochemical, medical, environmental, food, industrial, biosecurity or pharmaceutical analysis and personal diagnostics) which are based on connection of biological element or molecule with biological activity toward measured analyte onto surface of the used transducer [1,2]. The history of biosensor construction has started since half of the past century when Clark and Lyons invented the first enzymatic biosensor for detection of glucose from blood samples. Their work was based on artificial blood preparation therefore they needed the accurate measuring of blood analytes including glucose for

monitoring of organism homeostasis. Their intention culminated in development of a chronoamperometric platinum electrode that became the first invented biosensor in history. Since that, biosensors have gained their attention in scientific field for their high sensitivity, specificity and easy use. Their broad applicability in medical diagnosis and innovation, food safety and drug analysis or environmental monitoring predicts their phenomenal growth. As highly sensitive, robust and accurate devices are appropriate to use in everyday analysis tasks [3-5]. The current review is focused on the both survey of the actual trends and discussion of the expected forthcoming applications.

2. CONSTRUCTION OF BIOSENSORS

Biosensors are defined as analytical devices consisting of biological molecule giving specificity to analyte (it is also called biorecognition element) and physico-chemical transducer providing measurable signal working as a physical sensor [6,7]. Biorecognition element mediates selective biocatalysis or specific binding of analyte. Enzyme, antigen, antibody or nucleic acid usually belongs to one of recognition element and the specificity of measured system depends on it [8]. Biorecognition element is tightly bound onto physico-chemical transducer by physical or chemical immobilization methods. Transducers are able to measure signal arising from producing interactions and five groups of transducers are generally known: electrochemical and optical followed by mass-based are the most common but thermal and magnetic biosensors are well known as well [5,9,10]. Improving technologies allow development of novel, advanced and new designed transducers [11].

2.1. Biorecognition elements

Biorecognition elements can be divided into two groups: biocatalytical receptors including enzymes, whole cells, cell organelles, tissues and whole microorganism and bioaffinity receptors including antibodies, cell receptors or nucleic acids [9,12].

Biocatalytical receptors have been developed earlier because of easy applicability and they are based on transformation of biological reaction into a measurable physical value such as current, potential, fluorescence or spectral absorbance [8,13]. Biocatalytical receptors are usually connected with electrochemical, optical or thermometric transducers [13].

Enzymes are very common biorecognition elements mostly for their simple and well-known construction, high sensitivity, availability, satisfactory limits of detection and affordability. For instance glucose oxidase based glucose biosensors belong to the one of the most widespread type of enzymatic biosensors and it was also used in the first biosensor construction at all [9,11]. Enzymes normally transform substrate to corresponding amount of product which can be sensitively detected by optical transducer using appropriate chromogenic substrate. Oxidoreductases, the most relevant group of enzymatic biorecognition elements, catalyze oxidation or reduction reaction utilizing oxygen or cofactor while electrochemical signal is increasing. So the electrochemical transducers are also appropriate for application in enzyme-based biosensors. Enzymes can be immobilized on surface of a

transducer or tied to other biocomponent [11,14,15]. Some papers described multienzyme labeling system using two or more enzymes [16]. Poor long term stability, insufficiently high signal of amplification and problematic maintenance enzymatic activity of immobilized enzyme are the major drawbacks of the enzyme performance. Interferences of electroactive endogenous substances may also affect enzyme application in connection with electrochemical transducers [10,16].

Despite frequent application of enzymes as biorecognition elements, antibodies, nucleic acids and whole cells have got an attention in last decades. These bioaffinity receptors and sensors based on them work on principle of selective binding interaction between receptor and ligand where transducer enables to transform receptor-ligand interaction to measurable signal [9,15]. So the bioaffinity transduction can be beside other things performed by mass-based (piezoelectric) and magnetic transducers [13].

Antibodies have structure of immunoglobulins: two polypeptidic heavy and two polypeptidic light chains linked by disulfide bonds. On the grounds of heavy chains differences the five groups of antibodies have been discerned: IgG, IgM, IgA, IgD and IgE [17]. Antibodies have been used as biorecognition elements for last 20 years due to their broad spectrum of application, high specificity, sensitivity, selectivity and strong antigen-antibody interactions [9,10]. The biosensors having embedded antibody or working on antibody-antigen interaction are called immunosensors [15]. Monoclonal, polyclonal or recombinant antibodies are usually used in clinical practice and diagnosis. Polyclonal antibodies are secreted by multiple plasma cells, monoclonal antibodies are secreted by single clonal lineage and recombinant antibodies are produced during recombinant engineering by gene manipulation [17]. Often used monoclonal antibodies are produced by an immortal myeloma cells fused with spleen cells and they allow high specificity of antibody-antigen binding, homogeneity and production in unlimited quantities. But considerable drawback - immunogenicity may limit their use that´s why the chimeric, humanized, humanized recombinant and phage display antibodies were developed for decreasing the immunogenicity of murine monoclonal antibodies [18,19]. Antigens or antibodies can be labeled by enzymes, fluorescent or electrochemical compounds, radionuclides, or avidin-biotin complex because of inability of antibody-antigen complex to generate proper signal for optical and electrochemical transduction [20]. On the other hand, use of mass-based transducers converting mechanical deformation and voltage to measure mass or viscoelastic effects enables direct detection of arising bound without necessity of labeling [13]. The selectivity of measured system is determined by two identical very specific antigen binding sites on the molecule of immunoglobuline [18].

Deoxyribonucleic acid (DNA) is known as carrier of genetic information. It contains two antiparallel complementary polynucleotide strands consisted of purine and pyrimidine nucleotides linked by hydrogen bonds [15,21]. DNA as a biorecognition element is integrated on transducer surface as whole pre-synthesized probe (sequence of polynucleotide chain containing tens of nucleotides) or each base is immobilized on transducer surface individually [22]. DNA sensors (also called genosensors) are based on specific nucleic acid-analyte binding process like hybridization between targeting DNA and complementary probe and signal from hybridization is measured [21,22]. The hybridization probes are usually marked by fluorescent, electrochemical or radionuclide labels hence the electrochemical, optical or thermal transducers are normally applied nevertheless the most of

recent papers are focused on electrochemical detection. Small amounts of DNA have to be multiplied by some of the amplification methods such as polymerase chain reaction [8,22]. DNA sensors with construction based on nanomaterials belong to the one of the most clinical applied in last fifteen years. Nanomaterials based DNA sensors usually connected with electrochemical and fluorescent detection techniques are mostly used for detection of DNA sequences, hybridization and diagnosis of mismatches [23]. Generally, application of DNA sensors lies in food analysis and clinical diagnosis namely in inherited diseases and rapid infection diseases detection and screening of cDNA colonies [9,24,25].

Table 1. Summary of biorocognition elements, their advantages and disadvantages and transducers.

Biorecognition element	**Pros**	**Cons**	**Transducer**
Enzymes	Simplicity, well-known structure, sensitivity, affordability	Poor chemical, thermal and pH stability, often interferences	Optical Electrochemical
Antibodies	Broad spectrum of application, high specificity, sensitivity, selectivity and strong antigen-antibody interactions	Immunogenicity of polyclonal and monoclonal antibodies, price	Optical Electrochemical Mass-based Other (non-common type)
DNA	Wide spectrum of applications, high specificity	Necessity of labeling, time consuming related procedures for sample modification, price	Optical Electrochemical Thermal Other (non-common type)
Whole cells	Unnecessity of extraction and purification, long life-time, high pH and thermal stability, low cost, wide spectrum of different enzymes	Low selectivity, slow reaction	Electrochemical

Whole cells biosensors usually microbial (bacteria, fungi, yeast, algae, tissue culture cells) are based on connection of electrodes and immobilized living cell and on detection of organic compound assimilated/produced by cells. Advantage of these types of sensors is uselessness of extraction and purification, long life-time or high pH and thermal stability, microbial cells could be produced in large amounts and they contain a wide spectrum of different enzymes, on the other hand this type of sensors is slow and low selective [7,9,15].Using microbes as biorecognition element provides great capacity of acclimatization to given conditions, low cost unlike enzymes and antibodies and ability to mobilize new molecules [12]**.** Microbial cells are more stable and more easy to handle unlike plant or animal

cells [7]. Detection of the state of cells is based on electrochemical measurement of oxygen consumption/CO_2 production or on determination of changing redox potential or pH [26]. This type of biosensors has been usually applied in food analysis and environmental monitoring including heavy metals, pesticides or organic contaminants detection [9,12,15,25,26].

Summary of biorecognition elements, their advantages and disadvantages and the most often used transducers with specific type of element are showed in Table 1.

2.2. Immobilization techniques

Immobilization is either a physical or chemical method of entrapping the whole biorecognition or making an interaction of its part with the transducer surface. Selection of a suitable immobilization technique is one of crucial steps of sensor preparation because the possibility of biorecognition element inactivation caused by choosing inappropriate immobilization method is very high. Two types of immobilization techniques are generally known - physical and chemical Selection of more appropriate method depends on nature of the chosen biorecognition element, used transducer, physico-chemical conditions and properties of analyte [9,27-29]. Economic demands are also a criterion for selection.

Physical immobilization (Figure 1) is based on binding of biological molecules (most often enzymes) to transducer surface without creation of chemical bonds therefore physical entrapment, microencapsulation, adsorption and sol-gel technique belong to these immobilizations [28-32].

Physical entrapment is a method based on embodying biorecognition elements in three-dimensional matrices. A electropolymerized film, an amphiphilic network consisted of polydimethylsiloxane, a photopolymer, gelatin, alginate, cellulose acetate phthalate, modified polypropylene and polyacrylamide membranes or a carbon paste can be named as examples of entrapping matrices [28,31,32]. Electropolymerization is immobilization of biorecognition element mostly enzyme on electrode surface under applied current or potential in aqueous solution containing both biomolecule and monomer molecule (such as aniline, pyrrole or thiophene). Conducting polymerized film with precise spatial resolution over surfaces where the bioelement is entrapped inside is created [28,33,34]. Amphiphilic network is based on hydrophilic and hydrophobic polymers where biorecognition elements are anchored [35]. Soluble photo sensitive pre-polymer with crosslinking properties is another material often used for biomolecule immobilization during sensor construction. This soluble pre-polymer polymerized under light exposition to form of insoluble matrix. Alginate, the brown algae, is the most often used entrapping material due to its biocompatibility with bounded biorecognition element, non-toxicity and its sufficient accessibility for electrons, but other materials (such as gelatin, cellulose acetate phthalate, polypropylene, polyacrylamide) are also frequently used as the entrapping membranes. Carbon paste consists of graphite powder and pasting liquid and it makes up ideal substance connecting the entrapped biorecognition element to surface of transducer. It is usually used with electrochemical transducer [28,31].

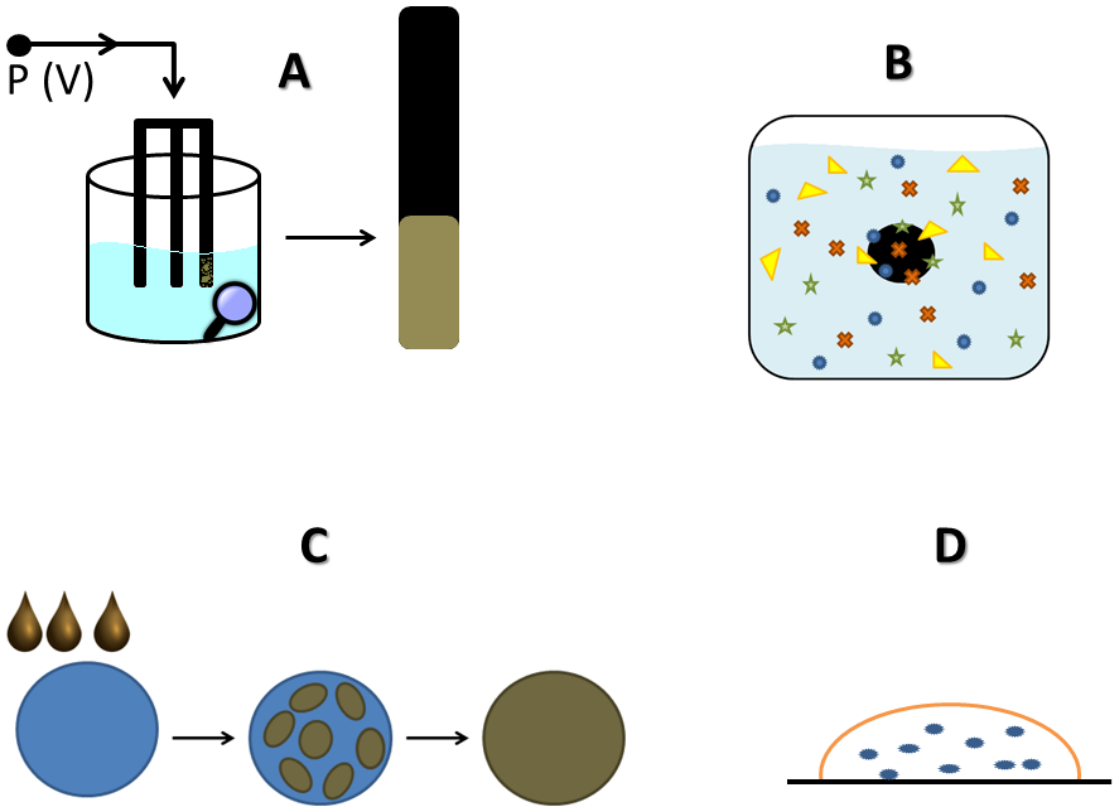

Figure 1. *Chosen types of physical immobilization.* A – Electropolymerization. Electrodes are submerged in electrolyte containing biomolecule and monomeric molecule is showed. While potential is affecting on the electrodes, polymer entrapping biomolecule is depositing on working electrode surface. B – Physical adsorption. Molecules spread in solution are bounded onto surface of adsorbant/transducer by van der Waals forces. C – Microencapsulation. Droplets of matrix containing biomolecule are spread on core surface, where matrix creates core cover. D – Entrapping of biomolecule into membrane. Molecules of biorecognition element are catch inside structure of solid amphiphilic network consisted of polydimethylsiloxane, photopolymer, gelatin, alginate, cellulose, acetate phthalate, modified polypropylene, polyacrylamide or carbon paste.

Physical adsorption is based on attaching of biorecognition element to the outside of inert material by van der Waals forces. This method has a lot of advantages such as the simplicity, great variety of materials and it does not require chemical modification of biological components. Despite that the clinical application may be limited by the possibility of biomolecule activity loss [9,36].

Sol-gel technique is based on low temperature forming of solid glass-like transparent film via hydrolysis and condensation of precursor alkoxide where bioelements are encapsulated. Extraordinariness of sol-gel membrane lies in its thermal and chemical stability, simplicity of preparation and possibility of large amount of biomolecule entrapment [37].

Chemical immobilization is based on creation of chemical bonds between functional group of biorecognition element (side chains unnecessary for its catalytic activity) and surface of the used

transducer. Chemical bonds are mostly forming on activated transducer surface carrying out by chemical reagents (such as glutaraldehyde or carbodiimide) or they are created directly because of pre-activated membrane applied on transducer surface. Covalent binding, and covalent cross-linking belongs to the chemical immobilization techniques [9,28,29].

Covalent binding is process where biorecognition element receives firm bond to either surface or inner cavity of membrane. It is the most widely used type of enzyme immobilization technique [38,39]. Process of immobilization through membrane matrix includes two steps: synthesis of functional polymer and covalent immobilization [38]. The binding process is based on reaction between functional protein groups (usually side chain of amino acids) of biorecognition element and reactive groups of transducer/membrane matrix surface [9]. Covalent binding provides increased lifetime stability and strong and effective bonding and it includes chemical adsorption (also called chemisorptions) and activation of carboxylic or amino groups [28,38].

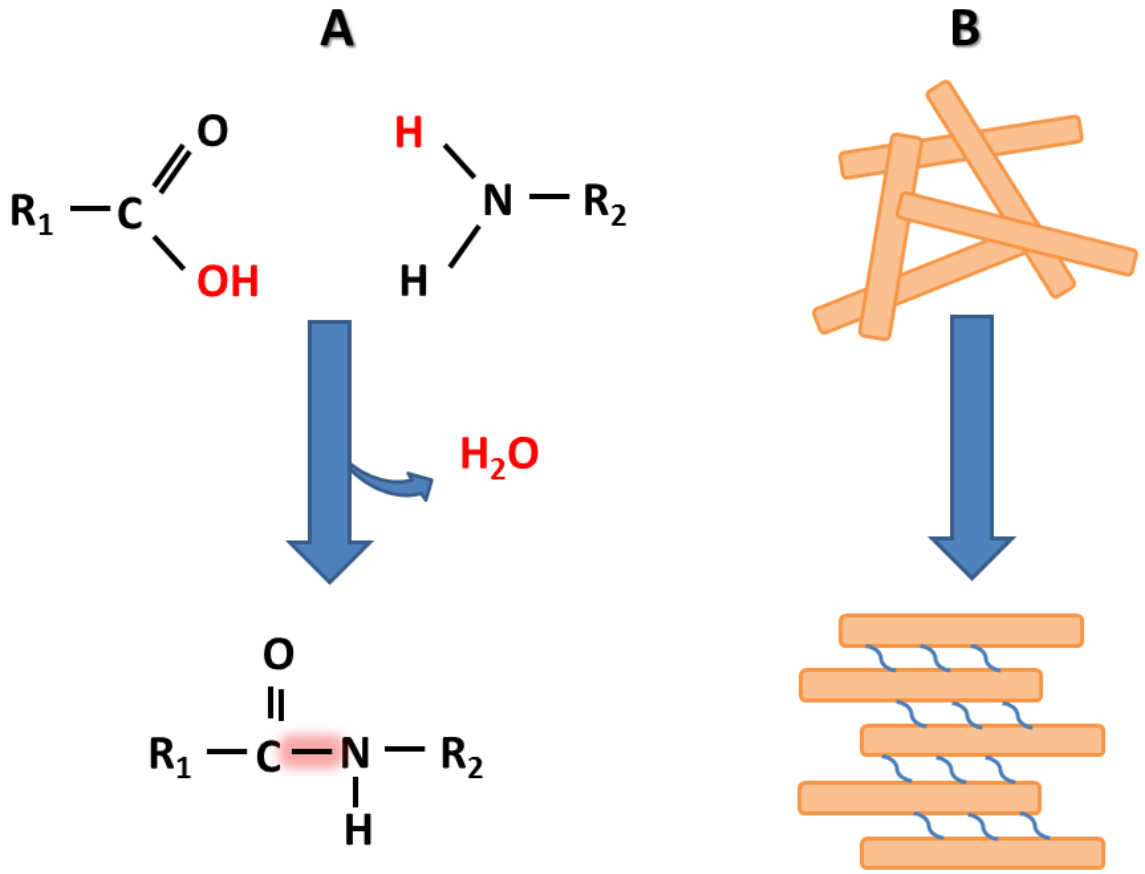

Figure 2. *Types of chemical immobilization.* A – Covalent binding. Creation of covalent bond between carboxylic and amino groups as an example of often chemical immobilization technique. R_1 and R_2 are representing the rest of biorecognition molecule and transducer surface/surface of carrier caught onto transducer surface. B – Cross-linking. Formation of three dimensional aggregates from single biomolecules (orange poles) via multifunctional linker (blue linkage) on transducer surface.

Cross-linking is an immobilization process based on covalent binding between biorecognition elements or between biorecognition element and functionally inert protein (for example bovine serum albumin). It leads to formation of three dimensional aggregates bonded via multifunctional linker molecule such as glutaraldehyde, glyoxal and hexamethylendiamine to the transducer surface [27,28,39]. Process of cross linking requires optimal conditions such as pH, temperature and ionic strength to allow shorter response time, stronger attachment and higher catalytic activity of enzymes [39]. Despite many advantages poor stability and partial denaturation of protein structure may limit application of cross linking immobilization [27,38]. Graphical survey of chemical immobilizations is given in figure 2.

Physical and chemical adsorptions together with covalent binding belong to the most used immobilization methods [9]. In the current time, high attention is given to magnetic nanosized and microsized particles as carriers of biorecognition elements. The particles are easily held on the transducer surface by an applied magnetic field and they can be washed out before further cycle by the magnetic field switch off. They can be easily processed in flow through devices which brings another advantage [40]. Adaptation in the field of biosensors is easy for the reason as seen in quoted examples [41-43]. Application of nanosized semiconductor particles like quantum dots is another option in the current biosensor technologies which provides good performance because of high quantum yields when fluorescence measured as an outputting value. Application of quantum dots in the biosensors construction is not an unknown technology when number of actual adaptations taken into consideration [44-46].

2.3. Tranducers

As was mentioned above five types of physico-chemical transducers can be distinguished. Beside other things transducers are vary according their construction, principle and possibility and frequency of their application. Electrochemical transducers have major role in diagnostic, optical transducers have important influence on research, but thermal, magnetic and mass-based transducers have not gained great clinical impact and nowadays they are use rarely [5].

The electrochemical transducers are based on monitoring of electric potential or electric current changes caused by electron or ions altering during biochemical reaction of biorecognition element (mostly enzyme) with analyte [47,48]. The enzyme transforms substrate to electroactive product creating measurable signal for electrochemical transducer [49]. On the other hand, other biorecognition elements are often connected with electrochemical transducer such as nucleic acid or antigen/antibody [12]. Electrochemical transducers can be divided into four groups according measured parameter on amperometric (electrical current), potentiometric (generated potential), conductometric (conductance of medium) and impedimetric (impedance of medium) devices [47,50]. Standard electrochemical sensor is composed from two (reference, working) or three (reference, working, counter) electrodes [12].

Amperometric transducers are based on measuring of current corresponding with amount of electroactive substance produced during chemical reaction in solution. Constant potential is set on

electrode so the measured current response to concentration of determined substance [9,50]. Only electroactive substances able to be oxidized or reduce can be determined by amperometric sensors [4]. Fastness, sensitivity, precision and linear response are advantages of amperometric determination over potentiometric devices. On the other hand, the interferences of another electoactive substances and poor selectivity belong to disadvantages of amperometric devices [9]. Potentiometric devices are based on measuring of potential changes between two electrodes (at near zero current) corresponding with amount of determined substance. Potentiometry is suitable for measuring of very low concentration or presented mass of analyte due to logarithmic device response on analyte concentration [50]. The fact that strong buffers can cause false negative finding is a disadvantage of the potentiometric biosensors. Impedimetric transducers are based on impedance changes after setting of small sinusoidal voltage excitation [4]. The impedimetric biosensors can be based on label free setup in some conditions [50]. Conductimetric transducers are based on changes of conductance between two electrodes because of chemical reaction. Impedimetric and conductometric devices are most often used at living biological system like microorganisms, organels and so on because inductance is decreasing and conductance is increasing during metabolic processes [4,9]. Electrochemical biosensors have been widely searched device for application in biotechnology, food industry, health care, medicine or environmental monitoring because they are ideal combination of highly sensitive electroanalytical device with selectivity of biomolecule [51].

Low cost, high sensitivity in miniaturized devices and possibility of measure turbid samples belong to benefits of electrochemical transducers which belong to one of the most applied type of transducers in biosensor construction [4,48,52].

Optical transducers such as fluorimeter, chemiluminescent detector, surface plasmon resonance and optical fiber can be applied in biosensors construction [22,50]. Optical transducers are often used for their high selectivity, sensitivity and fast detection of contaminants, toxins, drugs and microbial pathogens in water, bio-defence, environmental, clinical and food-safety analysis [48,53].

Fluorescence detection is based on emission of radiation during transition of valence electron from excited to ground state. Substances able to absorb light of sufficient energy, excite valence electron from ground to excited single state and emit photon at lower energy during reverse process are called fluorophore [47,48]. Chemiluminescent detection is performed using luminescent reaction of chemical substances such as luminol and its derivates (most commonly), it can be also performed by reaction catalyzed by biomolecule (hemin, peroxidase) or by application of potential. Luminescent reaction causes excitation of valence electrons and emission of radiation happens during transition of electrons to ground state [22]. Surface plasmon resonance is a non-linear optics based method using changes of light angle during its transition and electromagnetic interaction with a thin metal layer. Surface plasmon resonance detects the change of refractive index during binding of biorecognition element to surface of tranducer [50].

Optical fibers are traditionally cylindrical metal tube consisting of silica core usually doped with germanium and the core is surrounded by a cladding made from pure silica. Various types of modes of optical fibers are made according to their physical properties such as refractive index, core diameter and wavelength [54,55]. Optical fibers are based on their ability to conduct laser light to detected substance and to collect light emitted by excited substance [50].

Mass-based transducers are able to react to the minimal changes of substance weight binding on their surface. Those methods use small crystals (such as piezoelectric) which are able to vibrate at certain frequency depending on applied electric signal and on the mass of detected substance [47,50]. Piezoelectric, magnetoelastic and quartz crystal microbalance methods belong to mass-based transducers [22,47,50]. Mass-based transducer are very often used during detection of pathogens, antigens, antibodies or molecules enabling binding interactions [56,57]

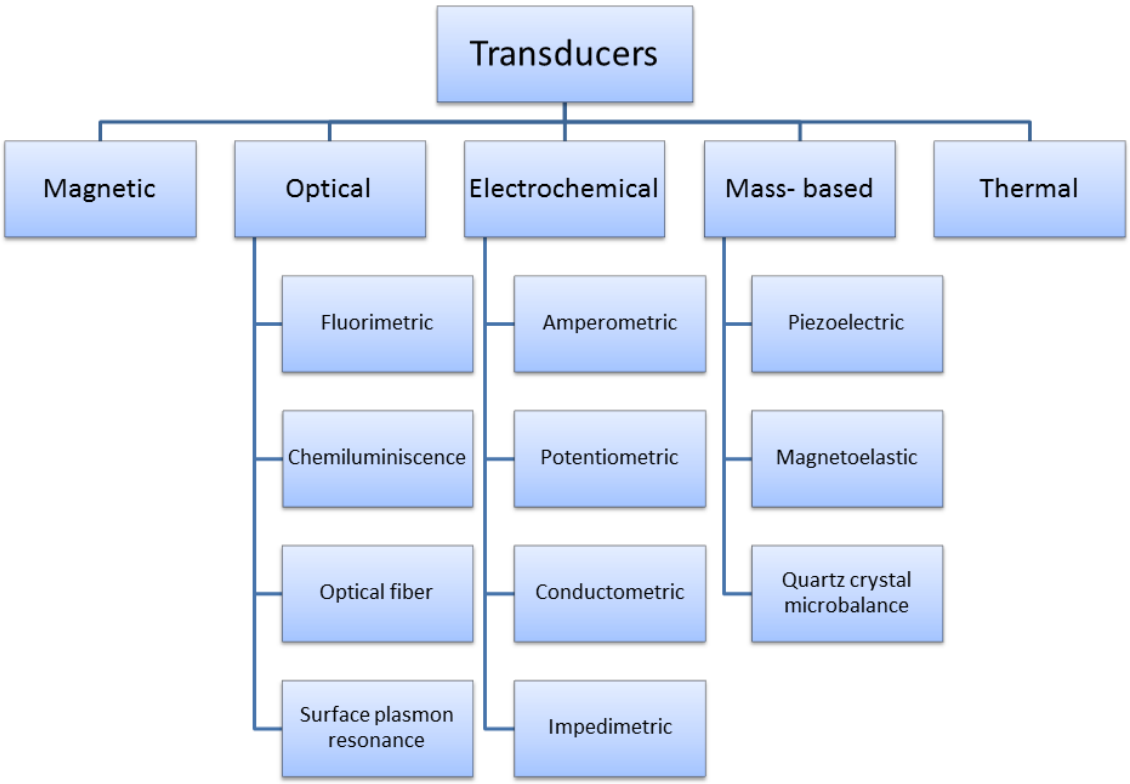

Figure 3. Overview of different types of transducers.

Piezoelectric biosensors are based on piezoelectric principle and they can generate and transmit acoustic waves of crystal oscillating at natural resonance frequency [4,48]. The decrease in resonant frequency signifies increasing weight of layer on transducer surface depending on interaction between biorecognition element and analyte [58]. In similar way the quartz crystal microbalance methods are based on piezoelectric principle because quartz crystal belongs to piezoelectric material. Quartz crystal makes up thin disc sandwich between electrodes deforming during application of electric field on electrodes. Resonant frequency of crystal hinges on amount of oscillating mass [22]. Piezoelectric biosensors belong to convenient real time detection method for monitoring of pathogenic microorganism [59]. Magnetoelastic sensors are composed of amorphous ferromagnetic ribbons with high mechanical tensile strength and their resonant frequency depends on length of sensor that´s why magnetoelastic sensors are based on detection of viscosity changes [47].

Thermal transducers are based on detection of temperature changes of circulating solution during biochemical reaction [60]. Usually the immobilized enzymes are used as suitable biorecognition element. Thermic reactions catalyzed by enzymes are measured by thermistor which consists of metal oxides beads or discs [61]. Survey of transducers is depicted in figure 3.

3. INTEGRATION OF NANOMATERIALS INTO BIOSENSORS CONSTRUCTION

Current research of novel biosensor construction is focused on nanotechnologies giving to them remarkable physical and chemical properties including the examination, handling preparation and use of materials and technologies under the size 100 nm. Nanomaterials enlarge surface area increase sensitivity and performance of sensors and provide high strength, excellent chemical reaction rate and unique electrical properties. The construction of nano-biosensors allows their integration into lab-on-a-chip devices [62-64].

Nanomaterials brought a new wind to the biosensors construction. Biosensors using nanomaterials for detection of analytes have showed higher sensitivity and lower detection limits. Moreover, nanomaterials proved their appropriateness for application in biosensing by their specific surface enabling immobilization of biorecognition elements increasing catalytic properties of sensors especially electrochemical ones [1,65]. Extraordinary electronic, optical, mechanical and thermal properties are characteristic for nanomaterials as well [66].

3.1. Nanostructures

Nanotechnologies such as nanoparticles, nanofibres, nanotubes, nanoprobes, nanorods, thin films etc. offer peerless magnetic, optical and electrochemical attributes implying fast, simple and multicomponent in vitro analysis.

Carbon nanotubes (CNTs) or multi-wall-carbon nanotubes (MWCNTs) usually represent application of nanotubes in biosensing. Since they have been discovered, CNTs or MWCNTs have gained the reputation of electrochemical modifier and attractive electrode support for enzyme immobilization because to their catalytic and sensitizing effect [67-69]. They have been emerging due to their ability to enlarge electrode active surface and to provide fast mass transport, good electrode accepting, fast electron transfer rates and good conducting [7,70].

Nanofibres have been emerging in ultrasensitive protein biosensor applications. They are extraordinary in this field of use for their high specific surface area, unique chemical structure and large pore volume per unit mass [65]. It can be also used in another type of biosensors such as carbon/ZrO_2/Cu nanofibres used as catalyst support for hydrogenation of pure CO_2 to methanol [71] or TiO2/CuO nanofibres for glucose detection [72].

Nanorods are often used as simple electrochemical modifier providing highly specific process [73]. They are usually prepared from gold, graphene, manganese, zinc or iron oxide or from combination of these materials. Detection of nucleic acid or basic biochemical markers such as glucose and hydrogen peroxide belongs to their most often application [73-78].

Nanoparticles have very similar application as all nanomaterials described above. They are appropriate for electrode modification where they increase the sensitivity and specificity of electrochemical catalysis. Moreover, nanoparticles, most usually magnetic or other metal, have been used as materials with pseudo-enzymatic activity able to provide catalysis of biochemical reaction on their own. They are named as mimics or mimetics. Peroxidase, very unstable enzyme with reduced lifetime, has been frequently replaced by the nanoparticles in construction of optical peroxidase sensor [79,80] or electrochemical peroxidase sensor [81,82]. Nanoparticles are also often used in optical sensors, where peroxidase is used as second enzyme for detection of substances such as glucose, cholesterol [83-86].

4. CURRENT APPLICATIONS OF BIOSENSORS

Huge field where different types of biosensors have been currently often applied is medicine and natural sciences. Wide spectrum of biosensor applications includes identification of microorganisms for determination of various diseases origin, identification of genome deviations for inborn defect assay or determination of biochemical markers indicated some metabolic disorders and pathological states of organism. As was mentioned in previous chapter nanomaterials and nanotechnologies are arising trend in biosensor applications and have been also often used in healthcare assays. They can be used for immobilization of enzymes, replacing unstable biomolecules as catalysts in reaction or for improvement of biosensor properties.

Novel electrochemical biosensor was constructed for determination of cholesterol levels by Satvekar and coauthors. Bioenzymatic nanobiosensor was based on DNA-assembled Fe3O4@Ag nanorods in silica matrix entrapping enzymes cholesterol oxidase and horseradish peroxidase onto surface of indium tin oxide electrode. Cholesterol levels were determined by cyclic voltammetry with limit of detection 5.0 mg/dl and linear range between 5.0 and 195 mg/dl [75].

Kostelnik and coauthors measured activity of AChE immobilized on magnetic particles by square wave voltammetry. They used screen printed sensor (carbon working electrode, platinum auxiliary electrode and silver chloride reference electrode) modified by Prussian blue for determination of reversible inhibitor of AChE tacrine with limit of detection equal 8.1 μmol/l [87].

Muralikrishna and coauthors designed an electrochemical sensor for glucose determination based on CuO nanobelts graphene composites (CuO@G) replacing enzymes. Methods such as X-ray diffraction studies, field emission scanning electron microscopy and transmission electron microscopy were used for characterization of composites and amperometry was chosen as detection device. According gained results CuO@G showed higher electrocatalytic activity for the oxidation of glucose than both reduced graphene and uncovered CuO nanobelts. Calibration curve showed linear response in the range from 0.5 to 6.5 μmol/l of glucose concentration and detection limit 0.05 μmol/l. The protocol has been successfully applied in clinic practice for analysis of human blood samples [88].

Huang and coauthors prepared sensor for human papillomavirus (HPV) DNA detection. Glassy carbon electrode was first coupled with graphene/Au-NRs (NRs = nanorods) and thionine was then electropolymerized on surface. Onto this platform, capture probe together with auxiliary probe was

hybridized for further DNA detection. Since DNA has negative charge, positively charged ion $[Ru(phen)_3]^{2+}$ (phen = phenantroline) was bounded for enhancement of electrochemical signal measured by differential pulse voltammetry (DPV). Limit of detection for HPV DNA 4.03×10^{-14} mol/l was reported [89].

Also non-common types of physico-chemical transducers have not been so rare in diagnostics. Crivianu-Gaita and coauthors described label-free real-time ultra-high frequency acoustic wave biosensor. Biosensor detected breast and prostate cancer biomarker parathyroid hormone-related peptide (PTHrP). Two different linkers – 11-trichlorosilyl-undecanoic acid pentafluorophenyl ester (PFP) and S- (11-trichlorosilyl-undecanyl) - benzothiosulfonate (TUBTS) – were apply for immobilization whole anti – PTHrP antibodies and Fab' fragments as biorecognition elements. Biosensor was optimized using X-ray photoelectron spectroscopy (XPS) and the ultra-high frequency electromagnetic piezoelectric acoustic sensor (EMPAS). Each linker was tested with no mass amplification and with sandwich-type secondary antibody mass amplification. The whole antibody-based mass-amplified biosensor shows the lowest limit of detection (61 ng/mL), a linear range from 61 ng/mL to 100 μg/mL and highest sensitivity. The Fab' fragment-based biosensor was used repeatedly. The whole antibody-based biosensor was able product analyte signal only at firs application [90].

Son and coauthors designed carbon nanotube (CNT) field-effect transistor (FET) biosensor. Biosensor detected symptoms of neuromyelitis optic aquaporin-4 (AQP4). AQP4 was been immobilized onto CNT-FET. Biosensor showed p-type FET characteristics after contact with AQP4, detection limit was 1ngl-1. AQP4 was detected without any pretreatment. Wang et al. developed sensor for detection GSH based on hybridization chain reaction of DNA probe. Hg^{2+} ions are bound in thymine-Hg^{2+}-thymine structure causes folding into hairpin structure. GSH can chelate Hg^{2+} which causes unfold of thymine-Hg^{2+}-thymine probe. This unfold ssDNA probe hybridizes with second probe immobilized on gold electrode surface. This dsDNA complex is labeled by $[Ru(NH3)6]^{3+}$ presented in grooves of dsDNA complex. Detection limit for GSH measured by chronocoulometric was calculated to be 0.6 nmol/l [91].

Wu and coauthors designed fluorescence resonance energy transfer biosensor for multiplex detection of pathogenic DNA based on gold nanorods. Biosensor was able to analyze multiple targets of DNA by one measurement. The limit of detection for the 18-mer, 27-mer and 30-mer targets was 0.72, 1.0 and 0.43nM. The recoveries of three targets were 96.57–98.07%, 99.12–100.04% and 97.29–99.93%. Method was simple, easy to manage and good selectivity [76].

Non-common type of electrochemical detection chose Hushegyi and coauthors for development of assay for detection of inactivated, but intact influenza viruses H3N2. Biosensor was based on impedimetric detection of glycan (viral receptor) – potentially pathogenic influenza bound. Receptor-ligand bound has great selectivity distinguished different subtypes of viruses. Limit of detection was set to be 13 viral particles in 1 μl [92].

Not only complicated and for preparation time demanding biosensors based on expensive procedures have been innovated. Such available device as smartphone camera can serve as a tool for assay performing. Pohanka used smartphone camera for detection of butyrylcholinesterase activity. In this paper, filter paper was soaked up with indoxylacetate and then butyrylcholinesterase solution was applied onto paper surface. Blue coloration of indigo blue was then shot by smartphone camera and

RGB channels were used for photography evaluation. Method was compared to standard Ellman´s assay using plasma and coefficient of determination 0.993 was achieved [93]. Similar array was described in another paper. Martinkova and Pohanka used smartphone camera for detection of color change for glucose assay. In this paper, bubble wrap as reaction container was used as another simple and low-cost material enabling simple and cheap determination of often measured blood glucose levels. The wrap was used for immobilization of glucose oxidase and peroxidase necessary for colorimetric detection of glucose and sol-gel membrane was used for immobilization of enzymes inside the bubbles. Method worked with reaction of enzymes, glucose and o-phenylenediamine dihydrochloride causing coloration. Final color replied concentration of glucose that can be easily log by cell phone. Limit of detection was considered to be sufficiently low (750 mmol/l) for blood glucose analysis [94].

Barring medical and healthcare assays biosensors found their place also in in pharmaceutical, food and environmental analysis.

Palanisamy and coauthors invented amperometric biosensor based on reduced graphene oxide (RGO) and polydopamine (PDA) composite for detection of chlorpromazine. Composites modified on glassy carbon electrode were prepared by electrochemical reduction of graphene oxide together with polydopamine. Assay showed linear range from 0.03 to 967 μmol/l and limit of detection was set to be 1.8 nmol/l. Composites also showed high selectivity toward chlorpromazine in presence of potentially interfering substances (metronidazole, phenobarbital, chlorpheniramine maleate, pyridoxine and riboflavin) and they showed appropriate recovery toward chlorpromazine in tablets form as well [95].

Weng and Neethijaran constructed microfluidic biosensor for detection of food allergens. Biosensor was based on quantum dots aptamer functionalized by graphene oxide complex and on conformation change of complex after interaction between biosensor and analyte which results in change of fluorescence. Method had linear dependency in concentration range from 200 ng/ml to 2000 ng/ml and limit of detection was equal to 56 ng/ml [96].

Bidmanova and coauthors innovated optical biosensor for monitoring of environmental pollutants namely halogenated contaminants in water samples. Method was based on reaction of enzymes with halogenated aliphatic hydrocarbons detected by change of fluorescence of pH indicator. Limit of detection for halogenated contaminants 1,2-dichloroethane, 1,2,3-trichloropropane or γ-hexachlorocyclohexane was equal to 2.7, 1.4 and 12.1 mg/l respectively. Small size, short time of measurement and portability was evaluated as the one of the biggest contribution of biosensor. Biosensor was compared with gas chromatography coupled with mass spectrometer and result showed biosensor is appropriate for use routine assay in both field screening and monitoring [97].

5. CONCLUSIONS

As was described above, nanotechnologies belong to one of the most innovated principles in novel biosensors construction. Different types of nanomaterial connected with unique transducing systems such as smartphone camera, frequency acoustic wave or fluorescence resonance energy transfer detection are highly invented and build in biosensors. Trend of our time is preparation of more

accurate and sensitive, faster and highly specific but on the other hand more complicated and financial demanding biosensors.

ACKNOWLEDGEMENT
A long-term organization development plan 1011 and Specific research funds MSMT SV/FVZ201605 (Faculty of Military Health Sciences, University of Defense, Czech Republic) is gratefully acknowledged.

CONFLICT OF INTERESTS
The authors declare no conflict of interests.

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