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Electrochemical Sensors and Biosensors for Influenza Detection – Literature Survey 2012-2013

Ludmila Krejcova[1,2], *David Hynek*[1,2], *Petr Michalek*[1,2], *Vedran Milosavljevic*[1,2], *Pavel Kopel*[1,2], *Ondrej Zitka*[1,2], *Marie Konecna*[1,2], *Jindrich Kynicky*[3], *Vojtech Adam*[1,2], *Jaromir Hubalek*[1,2], *Rene Kizek*[*1,2]

[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic, European Union
[2]Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic, European Union
[3]Karel Englis College, Sujanovo nam. 356/1, CZ-602 00, Brno, Czech Republic, European Union

[*]E-mail: kizek@sci.muni.cz

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This review summarized published information in the area of electrochemical detection of influenza virus in 2012 – 2013. The attention was mainly paid to summarize the news in the field of sensors and biosensors for influenza detection. Further, the impedance and quartz crystal microbalance sensing devices are also discussed.

Keywords: Influenza Virus; Electrochemical Detection; Biosensor; Sensor; Nucleic Acid; Viral Protein; Paramagnetic Nanoparticle; Voltammetry

1. INTRODUCTION

Because of new development in the assays for "pathogen determination and quantification", this review follows our previously published one focused on sensors and biosensors in the field of electrochemical detection of influenza virus [1]. Very fast development of this global research area leading to the application of new methods and materials in pathogen determination is obvious [2-4]. Therefore, the update of electrochemical detection approaches for influenza virus in the years 2012 and 2013 is summarized in this text.

Family of influenza viruses contains three genera: Influenza A, Influenza B and Influenza C. These three genera differ from each other in inside, species-specific nucleo-protein antigen, the number of gene segments, host specificity and clinical manifestations protein [5]. Because of the fact that the individual subtypes of influenza virus differ from each other by the variations in membrane virus constitution (membrane protein, ion channels, matrix proteins), various ways of detection based on individual parts of virus are investigated. Utilization of individual differences could help in detection of individual subtypes of influenza virus.

2. SENSORS

Our last review, which is updated by this text, was divided on two basic parts according to the way of virus determination. These two basic ways were called as sensors and biosensors. The difference in both approaches lies in the presence of bio-recognition element in the sensor construction. Developmentally, the usage of electrochemical sensors is an older way. Reasons for the course change from sensors to biosensors in the area of virus determination have been summarized [6,7]. Nevertheless, development in this area is still going on.

While the development in the application of mercury and amalgam electrodes is negligible, the usage of carbon and gold electrodes is widespread. Gold electrode has well defined surface and thus, these are very suitable for the electrode surface modification. This is probably the main reason why the latest investigation is focused to this electrode material. Particularly, modification of gold electrode surface through the chemical reagents [8,9] or magnetoimmunosensing entity [10,11] was published. Chemical modification is in this case presented by two approaches, where the first one is based on the functional architecture introducing receptor molecules as a sensing entity that mimics those found in the membrane of target cells of influenza A virus [8]. The artificial receptors are built by sequential assembly of 1-octanethiol/octyl-galactoside hybrid bilayer, followed by an enzyme-mediated functionalization of the terminal galactoside groups with sialic acid molecules. The detection mechanism relies hence on the specific affinity between the sialic acid-galactose receptor moieties anchored on the modified electrode surface and the hemagglutinin (HA) viral surface protein. In contrast to immunosensors based on antibodies as bioreceptor, the sialylated modified gold electrode is also able to distinguish among influenza phenotypes. The second way of chemical modification is represented by the diazonium salt as modifier of electrode surface [9]. More precisely, 4-carboxy phenyl groups located on the electrode surface were prepared for the indium tin oxide, gold and glassy carbon electrode. Modified glassy carbon electrode was tested for the functionality as influenza ODN hybridisation biosensor [9]. In addition, modification of gold electrode surface using magnetoimmunosensing entities was based on the connection of gold electrode with magnet and next usage of magnetic particles as catchers and carriers of influenza virus [10,11]. The electrochemical

response of suggested system was obtained from realized bienzymatic strategy. The first enzyme functional as tracer was tagged on immunomagnetic beads, which could be accumulated on the magneto controlled gold electrode and the second enzyme was immobilized on the electrode by layer-by-layer technique. This construction allowed obtaining the catalytically reduced electrochemical signal of H_2O_2 after the immunoreaction.

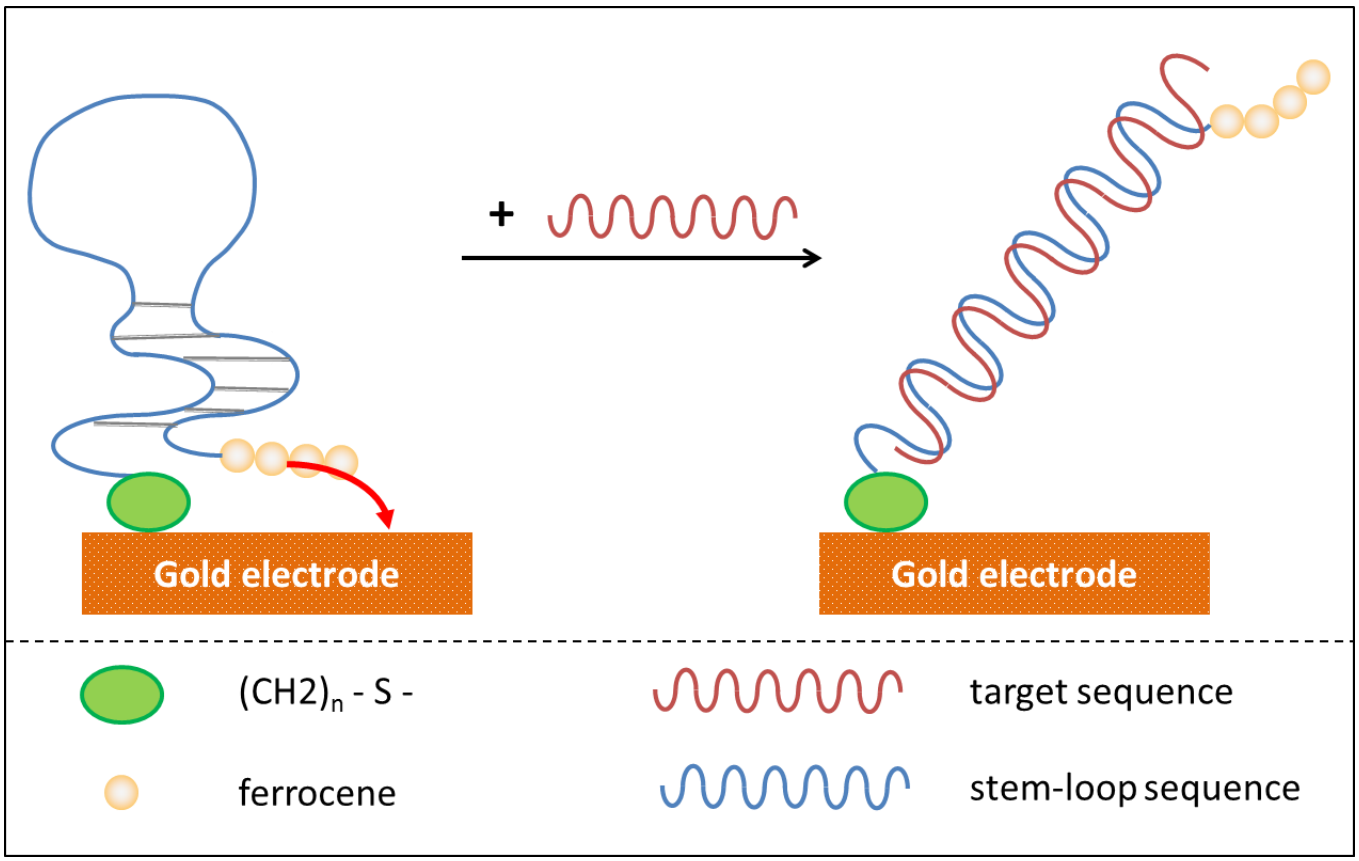

Figure 1. Schematic of the DNA detection system based on the stem-loop structured DNA probe. Probe was formed by the introduction of four ferrocene moieties at the 5′ end of a stem-loop oligonucleotide and a C6-thiol modifier group at the 3′ end. This 4Fc-DNA was immobilized on the surface of a gold electrode microsystem *via* standard thiol chemistry. Such architecture serves as sensor for DNA detection which is based on hybridization.

3. BIOSENSORS

3.1. Hybridisation on electrodes

ODNs belong to the first choice targets for formation of biosensing platform for pathogens. The simplest determined step is obviously hybridisation of target sequence. This procedure could be easily recorded by electrochemical methods. Well written overview of electrochemical real-time nucleic acid amplification was published by *Patterson et al.* [12]. This review is aimed on general pathogens quantification including influenza virus and divides presented information into four parts according the way of nucleic acid amplification as follows: solid polymerase chain reaction (PCR), solution-phase PCR using electrochemical reporters of product formation, solution-phase qPCR using sequence specific reporters, and isothermal amplification [12].

The newest ways of influenza determination based on hybridization reaction are connected with the application of electrochemical labels [13-19]. Usage of ferrocene as a modifier of specific ODN sequence was reported by *Chatelain et al.* [13]. They used four-ferrocene modified oligonucleotide at the 5'-end and a C6-thiol modifier group at the 3'-end as a probe for DNA detection with a gold electrode microsystem (Fig. 1). The probe sequence had a stem-loop structure that fold efficiently on the electrode, and thus optimized electron transfer. Such architecture served as sensor for DNA detection based on hybridization.

Grabowska et al. constructed sensor consisting of two different oligonucleotide probes immobilized covalently on the surface of one gold electrode (via Au-S bond formation) [14]. This sensor was used for simultaneous determination of two different oligonucleotide targets. One of the probes, bound on its S'-end with ferrocene, was related to sequence encoding part of hemagglutinin from H5N1 virus. The second probe, bound on its S'-end with methylene blue, was related to the fragment of neuraminidase from the same virus. Such sensor is able to detect main markers of the influenza virus, hemagglutinin and neuraminidase.

Our group published magnetic electrochemical bar code array for detection of single point mutations (mismatches in up to four nucleotides) in H5N1 neuraminidase gene [17]. Paramagnetic particles covered with dT_{25} were used as a tool for isolation of complementary H5N1 chains (H5N1 Zhejin, China and Aichi). For detection of H5N1 chains, oligonucleotide chains of lengths of 12 (+5 adenine) or 28 (+5 adenine) bp labelled with quantum dots (CdS, ZnS and/or PbS) were used. The obtained signals identified mutations present in the neuraminidase gene sequence.

3.2. Quartz crystal microbalance

Quartz crystal microbalance (QCM) contributed to the influenza research in last two years by two various ways. The first way is represented by the detection of influenza virus [20,21] and the second by the study of influenza virus binding capabilities [22,23]. Application of QCM for influenza detection was done through two various parts of influenza virus. In the first case, the aptamer was used for the formation of switch on/off system based on the crosslinked polymeric hydrogel [21]. A selected aptamer with high affinity and specificity against H5N1 surface protein was used, and hybridization between the aptamer and ssDNA formed the crosslinker in the polymer hydrogel. The aptamer hydrogel was immobilized on the gold surface of QCM sensor using a self-assembled monolayer method. The hydrogel remained in the state of shrink if no H5N1 virus was present in the sample because of the crosslinking between the aptamer and ssDNA in the polymer network. When it exposed to the target virus, the binding reaction between the aptamer and H5N1 virus caused the dissolution of the linkage between the aptamer and ssDNA resulting in the abrupt swelling of the hydrogel. The second part of virus used for detection was hemagglutinin, its binding capabilities, respectively [20]. *Diltemiz et al.* used 4-aminophenyl boronic acid as a new ligand for binding of sialic acid (having an important role in binding of HA) via boronic acid sugar interaction. QCM sensor surface was modified with thiol groups and then 4-aminophenyl boronic acid and sialic acid were immobilized on sensor surfaces, respectively.

Further studies of influenza binding capabilities were focused on hemagglutinin and its interaction. The first example of such study was the work of *Takahashi et al.* [22]. They studied the association of a sulphated galactosyl ceramide (sulphatide) with the viral envelope glycoprotein hemagglutinin. To determine whether the ectodomain of HA could bind to sulphatide, a secreted-type

HA (sHA), in which the transmembrane region and cytoplasmic tail were deleted, was applied. sHA showed subtype-specific antigenicity and binding ability to both sulphatide and gangliosides. Kinetics of sHA binding to sulphatide was demonstrated by QCM analysis. The second example was the work of *Wangchareansak et al.* focused on behaviour of N-acetylglucosamine [23]. N-acetylglucosamine is a part of the oligosacharide ligand responsible for the first binding step of virus (ligand-virus interactions) to a host cell. For immobilization on the gold surface, N-acetylglucosamine was linked to p-nitrophenol, and the nitro group was reduced and then bound to cysteine via two-step synthesis.

3.3. Nanoparticles

The main benefits of nanoparticle application lie in the improvement of electrode surface in the view of electrochemical reaction or better way of isolation procedure. Papers published in last two years aimed to these two ways. *Yanxia et al.* labelled antibodies by CuO NPs [24]. After the immobilization of the antibodies (attached on the solid substrate via physical adsorption between hydrophobic groups of antibody molecules and polystyrene), the CuO NPs were dissolved by adding acid to produce copper ions, which were electrochemically detected with high sensitivity and specificity. Another way of electrode surface modification was presented by *Jang et al.* [25]. In their work usage of ZnO nanorod network for the immunosensor fabrication was described. The immunosensor was evaluated in the acetate buffer solution containing 3,3',5,5'-tetramethylbenzidine (TMB) via cyclic voltammetry.

The improvement of the isolation procedure was described by *Kamikawa et al.* using electrically active magnetic polyaniline-coated nanoparticles as the transducer in an electrochemical biosensor for rapidly identifying influenza strains based on receptor specificity [26]. Electrically active magnetic nanoparticles were prepared by synthesizing aniline monomer around gamma iron (III) oxide (gamma-Fe_2O_3) cores, yielding 25-100 nm diameter nanoparticles. The nanoparticles were coated with monoclonal antibodies specific to H5N1. In addition, *Krejcova et al.* used glycan modified magnetic particles for isolation of influenza hemaglutinin and reported a new three-dimensional (3D), bead-based microfluidic chip for rapid, sensitive and specific detection of influenza hemagglutinin [19].

Gopinath and co-workers described immuno-AuNP assay, where gold nanoparticles were conjugated to an antibody against A/Udorn/307/1972 (H3N2) influenza virus to detect viruses on a sensing plate designed for an evanescent field-coupled waveguide-mode sensor [27]. One year later *Gopinath and coworkers* applied sensor based on previous experiment for demonstration that the anti-A/Udorn/307/1972 polyclonal antibody has the ability to discriminate between old and recently emerged influenza A/H3N2 viruses [28]. The authors were successful in the reaching of their aims. Moreover, *Bamrungsap et al.* report on a lateral flow immunoassay (LFIA) for influenza A antigen using fluorescently-doped silica nanoparticles as reporters [29].

Application of quantum dots for the influenza detection was presented by our group only. Different ways using quantum dots as labels were published [15,17,18,30,31]. Two main parts of influenza virus using for its detection was labelled by quantum dots, nucleic acids [16,18,31] and proteins (especially hemagglutinin) [15,30].

3.4 Aptamers

The utilization of aptamers for influenza electrochemical detection was published twice times in the defined time interval only [21,32]. The first experiment, published by *Wang et al.* [21], was described above in the section related to the QCM method. Another way of detection was based on the polymer microfluidic system with a functionalized conductive polymer microelectrode array [32]. *Kiilerich-Pedersen et al*. [32] show that DNA aptamer with affinity for influenza A virus (H1N1) were linked covalently to the conductive polymer microelectrodes in the microfluidic channel. The immobilization of aptamer on the electrodes provoked an increase in the impedance due to increased charge transfer resistance at the electrode/liquid interface. The binding of the virus to aptamers caused significant increase in the impedance already at very low concentrations. The washing the samples with PBS gave no significant change in the impedance signal, hence demonstrating that virus was bound to the aptamer.

3.5. Impedance

The utilization of impedance technique for the influenza determination had a great potential as it was shown in the last two years. Impedance determination was mainly connected with immunomagnetic separation [33-37]. Connection of immunomagnetic separation was studied mainly by the *Yan et al.* in four experiments differing in various technical designing of the experiments. They determined H5 subtype of avian influenza virus. In the first experiment [36], they used monoclonal antibodies against AIV H5N1 surface antigen hemagglutinin (HA), which were immobilized on the surface of gold microelectrodes through protein A for capturing influenza H5N1 in sample solutions. Electrochemical impedance spectroscopy was carried out in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe to describe the surface modification of microelectrodes and the binding of viruses. Other their experiments were connected with the application of immunomagnetic separation and differ in individual experiments design [33-35]. The first approach used streptavidin-coated magnetic nanobeads, where these particles were immobilized onto the biotin-labelled anti-H5 monoclonal antibodies to capture hemagglutinin (H5N1) from sample solutions by the specific immunoreaction. Then, these complexes were separated and concentrated by a magnetic field and the impedance magnitude was measured in a frequency range from 20 Hz to 1 MHz [35]. The second design was based on the modification of the surface of gold microelectrodes with protein A and then immobilization with monoclonal antibodies against an epitope in the hemagglutinin of H5 influenza subtype [34]. The binding of H5 subtype viruses onto the antibody-modified microelectrodes surface resulted in a change in the impedance, which was measured in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The third approach was based on the biotin labelled anti H5 monoclonal antibodies, which were immobilized onto the streptavidin coated magnetic nanobeads to separate and concentrate avian influenza virus H5N1 [33].

Except *Yan et al.* one other group published impedance biosensor for influenza detection. *Lum et al.* improved the impedance detection by labelling attached influenza H5N1 entities by chicken red blood cells for the impedance signal amplification [37]. The main principle of their assay is described in Fig. 2.

The application of impedance technique for influenza detection may not be combined with immunomagnetic separation only. Another approach for influenza impedance detection was published

by *Malecka et al.* [38]. They suggested a biosensor for detection of specific oligonucleotide sequences of H5N1 influenza virus. The NH2-ssDNA probe was deposited onto a gold electrode surface to form an amide bond between the carboxyl group of thioacid and the amino group from ssDNA probe. The signals generated as a result of hybridization were registered by impedance spectroscopy in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox mediator. This genosensor was capable to determine 180-bp (PCR products) oligonucleotides complementary sequences.

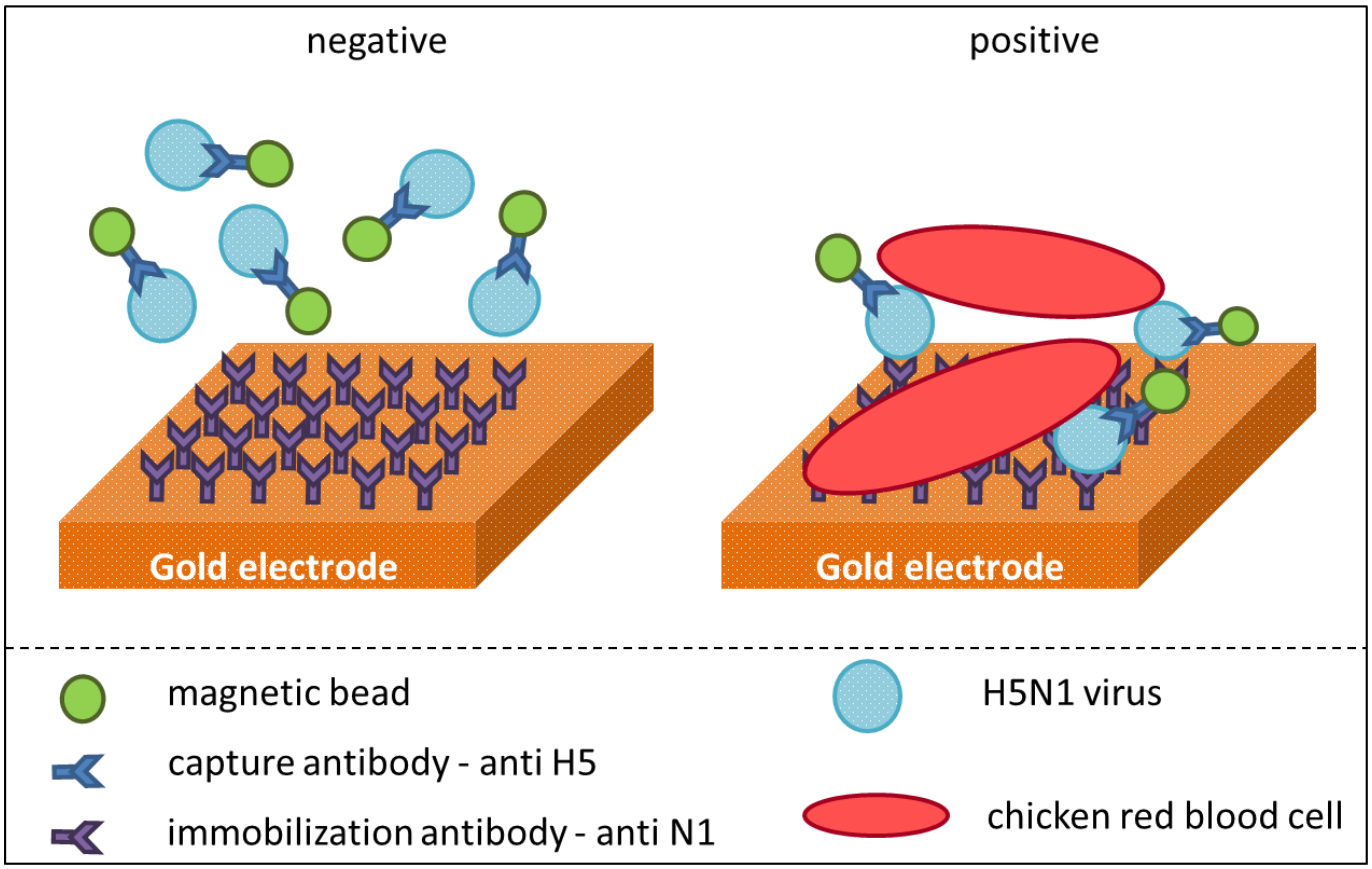

Figure 2. Design of non-Faradic impedance biosensor system using chicken red blood cells (RBCs) as bio-labels for improvement of impedance detection. A sample of influenza was isolated using immunomagnetic nanobeads coated with monoclonal antibody against H5. Second antibody against N1 subtype was interdigitated to microelectrode array. Target H5N1 virus was bound to the antibody on the electrode surface, causing a change in the impedance compared to a control sample. RBCs were used as biolabels to amplify the impedance change through their binding to the influenza virus on the electrode. RBCs were used as biolabels to amplify the antibody-virus binding due to their larger diameter (7-12 μm) compared to the virus (80-120 nm), and strong and specific binding by virus hemagglutinin to sialic acid linkages found on the cell surface. Both the virus and the RBC act as resistors in the system, and the RBC has a larger resistive value due to its larger size compared with the virus.

4. CONCLUSION

This review summarized published information in the area of electrochemical detection of influenza virus in the years 2012 – 2013. This text should be connection of our previous review

focused on this area of research [1], where interesting and promising seems to be aiming the attention at the impedance and QCM ways of influenza virus detection.

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Conflict of interest

The authors have declared no conflict of interest.

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