

Biosensor and Bioelectrochemistry Records in Bio-Analysis Lab at a Glance

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ABSTRACT

The Bio-Analysis Lab (with former name of Microanalysis Lab) was established in 1975 at the Institute of Biochemistry and Biophysics, University of Tehran. Many research works and studies have been carrying out in this lab such as electrochemistry of proteins, design and development of nanoparticles for development of novel biosensors, structural analysis of proteins by electrochemistry development of enzyme/microbial biofuel cells. For these purposes, different electrochemical and spectroscopic techniques and various imaging methods were used. In the present report, at first the direct electrochemistry of enzymes such as glucose oxidase, horseradish peroxidase, catalase, euphorbia latex amine oxidase, superoxide dismutase and horse heart cytochrome c were considered. Then, the methods for applying different types of macromolecules such as enzymes, antibodies, nucleic acids and aptamers for developing different types of optical and electrochemical biosensors were illustrated. At the end, some biocompatible nanoparticles for biomedical applications including cancer therapy and also application of some nanomaterials for improving the efficiency of biofuel cells were reviewed.

Keywords: Biosensor, Bioelectrochemistry, Electron transferring, Nanotechnology, Biofuel cells

INTRODUCTION

The Bio-Analysis Lab (with former name of Microanalysis Lab) was constituted in 1975 at the Institute of Biochemistry and Biophysics, University of Tehran. Our main research areas are concentrated in three main fields: direct electron transfer of redox proteins, designing optical and electrochemical biosensors, and synthesis of biocompatible nanoparticles for biomedical applications.

The direct electron exchange of proteins with electrode surface confronts with some problems due to their large and complex structures. In addition, the redox active centers of enzymes are surrounded by different functional group which limit their accessibility. To overcome these limitations, the electrodes can be modified either by mediators to communicate with proteins or be covered by a film containing protein [1]. The mechanistic study of direct electrochemistry of redox proteins is important because of providing a proper model for intuition the electron transfer in bio-systems. The results of these studies can be employed to design the efficient electrochemical biosensors and devices [2].

The technology for developing a biosensor is usually derived by integrating the knowledge resulted from different fields of science and engineering. This technology leads to design and construct the biosensors for applying in different areas including human health care, agriculture, industry, environment and military purposes. The biosensor is commonly described as a device in which a bio-receptor molecule intimately couples with a physical transducer to transform the biological response as a function of an analyte concentration into an electrical signal. Three major components of biosensors are bio-receptor, physical transducer and processor. The bio-receptor may be proteins, enzymes, antibodies, or nucleic acids that is incorporated with physical transducer *via* immobilization [3,4]. The intelligence in a biosensor is originated by the specificity of the bio-receptor toward the target molecule. Transducer is a critical part of a biosensor, which can be based on various sensing parameters such as temperature, potential, current, impedance, mass, and light transmission [5,6]. Biosensors usually decrease the steps for sample analysis and consequently reduce the analysis time and cost. This is why we have been keen to develop the novel biosensors with high specificity and selectivity for various applications.

Herein, we overview the published articles which have

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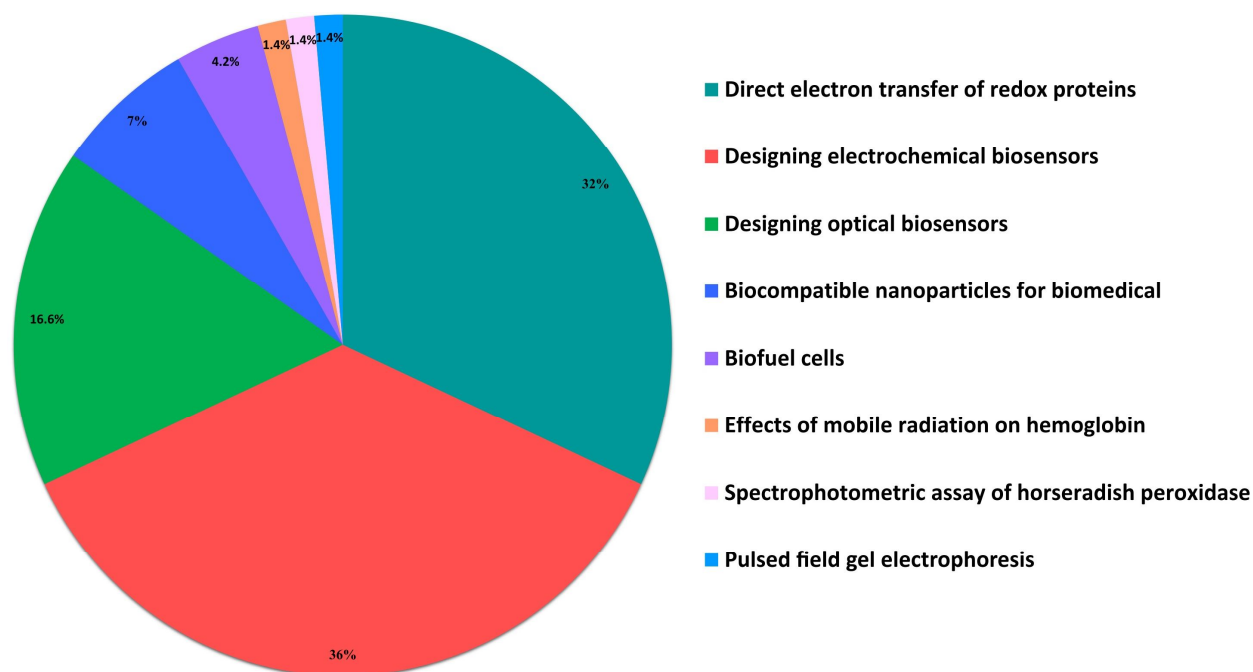


Fig. 1. An overview of the research topics carried out in Bio-Analysis Lab.

been carried out in our lab which were mainly published in the following research areas, as shown in Fig. 1: direct electron transfer of redox proteins (32%), designing electrochemical biosensors (36%), designing electrochemical biosensors (16.6%), biocompatible nanoparticles for biomedical applications (7%), pulsed field gel electrophoresis (1.4%), spectrophotometric assay of horseradish peroxidase (1.4%), and effects of mobile radiation on hemoglobin (1.4%), and biofuel cells (4.2%). We honored that the results of our researches were published mostly in qualified international journals with relatively high citations.

DIRECT ELECTRON TRANSFER OF REDOX PROTEINS

In order to find the mechanism governing direct electron transfer of redox proteins, we have studied some proteins which play important role in biosensor development. In 2006, we studied the direct electron transfer of four redox proteins including horseradish peroxidase (HRP), euphorbia latex amine oxidase (ELA), superoxide dismutase (SOD),

and horse heart cytochrome c (Cyt c), at gold electrode modified by Nafion-cysteine functional membrane [7]. The Nafion-cysteine acted as a promoter between protein molecules and the gold electrode. The redox response of proteins with medium molecular weight (HRP and SOD) was efficient on the Nafion-cysteine membrane, while proteins with very low or very high molecular weight (Cyt c and ELAO) demonstrated weak redox response. In 2007, a novel Nafion-riboflavin functional membrane was fabricated as a mediator for facilitating the electron transfer rates of Cyt c, SOD and hemoglobin [8]. The results showed that the modified electrodes did not decrease the activity of proteins after immobilization. We also had reported that covalent attachment of anthraquinone 2-carboxylic acid (AQ) to the surface-exposed Lys residues of HRP caused the direct electron transfer of the enzyme with a conventional electrode [9]. Further studies in molecular dynamics simulations revealed that modification HRP with AQ facilitates the accessibility of His-42 and heme prosthetic group to hydrogen peroxide (H_2O_2) and aromatic substrates. To improve the sensitivity and reproducibility of biosensor toward H_2O_2 , the Nafion-cysteine functional

membrane was applied as a promotor for increasing the HRP electron-transfer rate [10]. In 2008, considering the ability of proteins to react with Woodward's reagent K (WRK), we applied this method for chemical modification of catalase (Ct). The WRK-Ct-Nafion film on the glassy carbon electrode (GCE) led to a direct electron transfer between the enzyme and electrode and showed electrocatalytic activity toward H_2O_2 [2]. The modification of catalase along with the oxidative treatment of the electrode prohibited the adsorptive denaturation of the protein via decreasing hydrophobic interactions between the enzyme and electrode surface. In 2009, the direct electrochemistry of glucose oxidase (GOx) was surveyed on carbon nanotubes (CNTs) grown on Fe/MgO catalyst layer deposited on the alumina substrate [11]. Fe/MgO was a catalyst for growing CNT strands on the alumina substrate. CNTs were able to increase the conductivity of electrode and promote electron transfer between the electrode and GOx. The proposed biosensor was successfully applied for glucose sensing. In 2010, direct electrochemistry of catalase was obtained by confining the protein within multiwall carbon nanotube (MWCNT)-dodecyltrimethyl ammonium bromide (DTAB) film covered with a layer of Nafion (NF) (MWCNT-DTAB-Ct/NF) on the surface of a GCE [12]. The MWCNTs changed the irreversible reduction peak of catalase in DTAB-catalase/NF film to a pair of redox peaks. In addition, the film could determine H_2O_2 without mediator. In another study, a GCE was covered by a thin layer of polymethylen green (PMG) and then carboxylic acid functionalized carbon nanotubes [13]. Thereafter, enzyme was immobilized on the electrode surface as following steps: three layers of PMG, alcohol dehydrogenase and PMG were added to the modified electrode. The presented method could be used for development of biosensor for alcohol detection.

In 2010, a nanocomposite material consisting of amine functionalized TiO_2 -coated carbon nanotubes was prepared and used for GOx absorption [14]. The GOx bearing nanomaterial was fixed on a GCE to construct a novel biosensor for glucose determination. The direct electrochemistry GOx at modified GCE was reported. The modified electrode showed good capability and stability for determination of the enzyme electron transferring parameters.

In 2011, we identified the role of anticancer palladium complex in release of Cyt c from the biological membrane *via* assembling the artificial monolayer on the surface of a gold electrode to adsorb Cyt c [15]. The electrochemical and spectroscopic studies disclosed a conformational change after bounding of Pd complex to Cyt c which in turn caused the release of Cyt c from the model membrane.

The electrochemical and electrocatalytic behavior of choline oxidase (ChOx) on both single wall carbon nanotubes (SWCNTs) and MWCNT at GC electrodes were studied [16]. The results revealed the better carboxylic groups functionalization, higher electrical conductivity, better reversibility of the enzyme redox reaction, and higher electron transfer rate on the MWCNTs modified GCE. On the other hand, ChOx on the nanocomposite consisting of a room temperature ionic liquids (RTIL) and SWCNTs showed higher enzyme-substrate affinity, lower detection limit, higher sensitivity and more extended linear range toward choline. When GOx immobilized on GCE *via* a synthesized nanocomposite containing amine functionalized MWCNTs and a room temperature ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate), the enzyme direct electron transfer to the electrode was facilitated obviously [17].

In 2013, the interaction between HRP and gold-coated magnetic nanoparticles (GMNPs) and silver-coated magnetic nanoparticles (SMNPs) were surveyed and GMNPs were found more suitable for preserving structure and function of HRP than SMNPs [18]. In fact, silver surface in SMNPs facilitated oxidation and generation of free radical. In another research, we investigated the effect of osmolytes on the electron transfer characteristics of catalase as a key antioxidant enzyme [19]. The results revealed that proline as a compatible osmolyte increases electron transfer, while histidine as a non-compatible one reduces it. In another work, it was observed that compatible osmolytes including proline, xylitol, and valine destabilize the denatured form of the catalase, so, elevate its enzymatic activity, disaggregation, and thermal stability [20]. On the other hand, histidine could stabilize the denatured form of the protein and as a result, decrease the thermal stability and enzymatic activity of the catalase. The ability of MWCNTs and MWCNTs-([BMIM]BF₄) for investigation of direct electrochemistry of GOx and hemoglobin and improving the

electron transfer rate between proteins and electrode surface was compared [21]. Also, the structure of hemoglobin or GOx conjugated with supramolecular network of the nanocomposite showed to be maintained better than those absorbed on MWCNTs. In another study, the direct electrochemical properties of immobilized hemoglobin on the carboxyl functionalized MWCNTs/cysteine/AuNPs-modified GCE were surveyed [22]. Then, this nanocomposite was successfully used for determination of low concentrations of H_2O_2 . In 2014, the direct electrochemistry of hemoglobin was studied by entrapment in the surface of Ce-Cu-Zr nanocrystalline mixed metal oxide carbon paste electrode using an alginate biocompatible biopolymer that crosslinked with the calcium ions [23]. The molecular docking and experimental studies were used for studying benzene interaction with Hb. The results showed that benzene interacts with Hb in both globin and hydrophobic pocket regions of Hb. Also, it was suggested that the proposed electrode can be utilized as a new inexpensive electrochemical sensor for benzene. Also, we improved the direct electron transfer of human adult and fetal hemoglobin (HbA and HbF) on the surface of nanocomposite containing the carboxylated MWCNTs and a hydrophilic RTIL [1]. Moreover, the nanocomposite could differentiate between HbA and HbF based on their different electrochemical and catalytic behaviors *via* better analytical characteristics of HbF immobilized on the MWCNTs-RTIL/GC electrode toward H_2O_2 . Another development was performed to improve direct electrochemistry of GOx and increase the sensitivity and selectivity of glucose detection [24]. To this purpose, a novel GOx-hydroxyl fullerenes nano-complex was self-assembled and immobilized on a GCE followed by chitosan covering. We also synthesized CMWCNTs/Cys/AuNPs nanocomplex as a promoter and bridge between the electroactive center of an HRP molecule and GCE [25]. The modified GCE could be employed as H_2O_2 biosensor with good sensitivity and stability. In 2015, we developed a rapid method for constructing glucose electrochemical biosensor by covalent attachment of GOx to gold-coated magnetic iron oxide nanoparticles (Fe@Au) [6]. The direct electrochemistry of GOx showed a quasi-reversible cyclic voltammogram corresponding to the flavin adenine dinucleotide (FAD/FADH₂) redox couple. In 2016,

the effects of different types of the CNTs' functional groups were used to investigate the electrocatalytic behavior of ChOx at RTIL/CNTs nanocomposites [26]. The results revealed that NH_2 -CNTs provides more suitable environment for ChOx activity rather than $HOOC$ -CNTs because of the longer NH_2 chains. So, the ChOx/RTIL/tetraethylenepentamine (TEP)- NH_2 -CNTs biosensor was introduced more suitable for biosensing applications rather than other prepared enzyme electrodes.

Designing Electrochemical Biosensors

In 2005, HRP-modified electrodes which is mostly applied for measuring the amount of H_2O_2 , was constructed [27]. In order to provide fast electron transfer junction between the electrode surface and the redox center of peroxidase, the anthraquinone 2-carboxylic acid (AQ) was used as a novel electron shuttling mediator. Cyclic voltammetric results indicated that dissolved AQ molecules can transfer electrons between the heme site of HRP and the GCE. Moreover, covalent attachment of AQ molecules to the lysine residues of HRP revealed a direct electrical relationship between the modified enzyme and the conventional electrode. The modified electrodes showed high current responses to H_2O_2 .

In 2007, the iodide-modified silver electrode was used to find the affinity of the 2,3-diphosphoglycerate (DPG), inositol hexaphosphate (IHP), guanosine 3',5'-tetraphosphate (GTP), guanosine diphosphate (GDP), and guanosine monophosphate (GMP) to Hb [28]. The results revealed that DPG and IHP have one specific binding site on Hb, while GTP has multiple binding sites. However, GTP had weak effect on Hb. The no change of the cathodic and anodic peaks of Hb in the presence of GDP and GMP showed the lack of binding to Hb. In another report, a bromide-mediated silver electrode was applied to the measurement of Hb [29]. The bromide mediator showed very strong redox behavior with the silver electrode. In the presence of pure Hb, the modified electrode showed positive shift in cathodic and anodic peaks of bromide. Also, a bromide-modified silver electrode was applied to compare the redox potentials of different metalloproteins and differentiating the metallo- from non-metalloproteins [30].

The positive potential shift of the bromide ions was observed with addition of myoglobin, cytochrome c and catalase to the solution. The change in the cathodic peak current was seen for bromide ions in the presence of SOD, while such alteration was not observed in attendance of albumin. In another work, a polyorganic nanocomposite containing nanoparticles of methylene blue and Nafion was fabricated [31]. The meshy protuberances and hollow structures simplified absorption of HRP to sensitive determination of hydrogen peroxide. Also, an electrochemical nanoparticle-based method was used for hepatitis B virus (HBV) DNA detection [32]. The assay consisted a probe extending on magnetic beads, binding of streptavidin-coated gold nanoparticles to the hybridized biotin-labeled target sequences, silver enhancement, and chronopotentiometric detection of silver at gold electrode.

In 2009, a simple mono-enzyme biosensor was constructed for specific determination of paraoxon (POX) [33]. To reach this aim, the screen-printed electrode was modified by Prussian blue (PB) and ChOx for detection of POX as inhibitor. The concentration of H_2O_2 produced by ChOx was electrochemically determined by the PB modified electrode and decline in current intensity due to the addition of inhibitor. Also, a nanoparticle-based electrochemical method for detection of HBV DNA sequences was developed based on the adsorption of amplified HBV DNA strands on the probe-coated paramagnetic particles and electrochemical detection of hybridized strands using a hanging mercury drop electrode [34]. In another study, a homogeneous monolayer of Cyt c with appropriate orientation was constituted through the strong electrostatic and covalent interactions on a self-assembled monolayer C_{11} -modified electrode, which led to remarkable increase in electron transfer rate constant and stability for Cyt c [35].

In 2010, a biosensor for detection of H_2O_2 was designed based on immobilization of anthraquinone 2-carboxylic acid modified HRP [36]. Direct electrochemistry of the modified enzyme exhibited a quasi-reversible voltammogram. The nanocomposite of amine functionalized MWCNTs and a 1-butyl-3-methylimidazolium tetrafluoroborate was also synthesized and applied to construct a novel catalase based biosensor for detection of H_2O_2 [37]. The direct electron

transfer between catalase and the underlying electrode was improved in the presence of nanocomposite film, which led to efficient H_2O_2 determination. Also, the H_2O_2 produced by two gamma emitter radioisotopes of thulium-201 and technetium-99m was measured by anthraquinone 2-carboxylic acid (AQ) modified HRP [38]. In another work, SOD was immobilized onto silicon carbide nanoparticles to construct an efficient superoxide biosensor [39]. In order to develop a new organophosphorous sensor, prussian blue was electrodepositionized on a graphite electrode as a template for immobilization of Chox along with Nafion and bovine serum albumin [40]. The results showed the applicability of designed biosensor for measurement of paraoxon and ethyl parathion.

In 2011, a nanocomposite including the carboxylated MWCNTs and 1-Allyl-3-methylimidazolium bromide (AMI-Br) as an RTIL was synthesized which could serve as a proper host matrix for Cyt c [41]. The prepared nanocomposite could improve the direct electron transfer of Cyt c and showed specific behavior toward O_2^- which makes it suitable candidate for commercial purposes. Also, cysteine self-assembled monolayer-modified gold (Cys/Au) electrode was utilized to immobilize SOD and investigation of the direct electron transfer between enzyme and electrode surface [42]. The analogues between voltammograms of different electrodes indicated that the increase in current intensity by the order of $Cu^{2+}/Cys/Au > SOD/Cys/Au > Cys/Au$. In the same year, the reaction mechanism for chemical modification of tyrosinase by Woodward's Reagent K and its covalent attachment to a GCE were investigated [43]. The results showed the possibility of enzyme direct electrochemistry and then development of a biosensor for caffeic acid and L-3,4-dihydroxyphenylalanine (L-DOPA) detection. Moreover, the nanocomposite of amine functionalized TiO_2 -coated MWCNTs was synthesized by sol-gel method [44]. Then, it was applied for one-step immobilization of GOx to sense glucose. In another study, nickel oxide nanoparticles provided the proper adsorption sites for SOD which led to facilitation of electron transfer without using any mediators or promoters [45]. This nanocomposite was further used to detection of superoxide. Also, we developed a sandwich-like biosensor to diagnosis human immunoglobulin G (HIgG). We applied amine functionalized MWNTs (MWCNT-NH₂) to react

with the aldehyde groups on anti-IgG [46]. Then, the HRP conjugated anti-HIgG was used as the second antibody for signal production. The HRP could generate electrochemical signal in the presence of HlgG target and also H_2O_2 and potassium iodide as substrates.

In 2012, an artificial peroxidase (AP) was synthesized using the complex comprising hemin, imidazole Gemini and sodium dodecyl sulfate (SDS) immobilized on a nanocomposite consisting of carboxyl functionalized multi-walled carbon nanotubes (MWCNTs-COOH) and gold nanoparticles (AuNPs) [47]. The nanocomposite on GCE could be applied for H_2O_2 determination. Also, six different nano-composites containing the same amine functionalized multi-walled carbon nanotubes (NH_2 -MWCNTs) but different RTILs were synthesized to be used for studying the electrochemistry and electrocatalysis of ChOx [48]. The results showed the dependency of the electrocatalytic activity and the electroanalytical performance of immobilized ChOx on the degree of hydrophilicity of RTILs used in the nanocomposite.

In 2013, catalase was immobilized on the functionalized MWCNTs and L-cysteine modified gold nanoparticles for determination of the hydrogen peroxide [49]. In 2013, two biosensors were developed to detection of HBsAg. In the first work, the biotinylated hepatitis B surface antibody was immobilized on streptavidin magnetic nanoparticles and used for targeting the HBsAg [4]. By the addition of HRP conjugated with secondary antibody (HRP-HBsAb), an immunoassay sandwich was constituted. As a result, aminophenol as substrate for conjugated HRP was enzymatically changed into 3-aminophenoxazone (3-APZ) proportional to the HBsAg concentration, which was monitored by cyclic voltammetry. Also, a capacitive immunosensor was developed *via* covering the gold electrodes by an insulating layer of 3-ethylene glycol alkanethiols and then immobilization of the primary antibody [50]. The distance between the capacitor plates and the average surface area of the plates was changed after addition of HBsAg, which caused an alteration in capacitance. In the next research work, we applied dielectric barrier discharge (DBD) plasma treatment as a physical method for amine-functionalization of MWCNTs (NH_2 -MWCNTs) [51]. The GCE modified by NH_2 -MWCNTs was employed for immobilization of GOx and thereafter

successful oxidation of glucose.

DESIGNING OPTICAL BIOSENSORS

In 2010, we showed that the chemiluminescence technique has the ability to be used for direct detection of gamma rays and evaluating the dose rate of rays irradiated to watery substances [52]. It was obtained by production of H_2O_2 in a luminol alkaline solution by two gamma emitter radioisotopes of ^{201}Tl and ^{99m}Tc . The determined H_2O_2 concentration was correlated with the gamma ray detection and dosimetry. In another study, the electrochemical and chemiluminescence methods were successfully used to detect the H_2O_2 produced by gamma emitter radioisotope of cobalt-60 (^{60}Co) [53]. So, both proposed techniques, with leaner range from 0.25-5 Grays are applicable for detection and dosimetry of gamma ray from ^{60}Co . Also, we used a gamma rays emitted by a ^{60}Co to synthesis the three-dimensional hybrid nanomaterial of graphene-multiwalled carbon nanotubes (G-MWCNTs) for amperometric sensing of glucose in alkaline solution [54].

In 2014, the chemiluminescence technique was used to follow up the H_2O_2 production during hemoglobin fructation [55]. After accumulation of H_2O_2 , heme was degraded; so, it was concluded that fructose can have key role in H_2O_2 production, proteins glycation, and heme degradation during diabetes. Also, HBsAg was detected by immune sandwich comprising primary antibody, immobilized in polystyrene wells and co-immobilization of secondary antibody and luminol on the gold nanoparticles [56]. The detection signal was luminescence produced in the presence of hydrogen peroxide as an oxidant agent and Au^{3+} as an efficient catalyst for luminol oxidation. In another work, a sandwich type immunoassay system including the specific coupling of multi-functionalized gold nanoparticles bearing biotin and luminol molecules to the streptavidin modified by secondary antibody was designed [57]. Then, the chemiluminescent signal generated by the gold nanoparticles in the presence of $HAuCl_4$ as catalyst and H_2O_2 as oxidant was followed. In another research, the hydroxy naphthol blue was used as metal indicator for monitoring of rolling circle amplification (RCA) reaction *via* the changes in the concentration of soluble Mg^{2+} [58]. The proposed procedure was utilized for determination of real H5N1 samples. Also, it can be

employed either as a quantitative or qualitative biosensors. Afterwards, we developed another isothermal amplification method for the sensitive detection of the H5N1 influenza virus [59]. The padlock probe specifically was bound to the H5N1 target and circularized with T4 DNA ligase enzyme. After that, this circular probe was amplified by hyperbranched rolling circle amplification (HRCA) using Phi29 DNA polymerase. The detection method was based on the fluorescence intensity recorded at different intervals by intercalation of SYBR green molecules into the double-stranded product of the HRCA reaction. In another study, a soft-template nanostructured peroxidase (SP) was constructed through mixing Cyt c with SDS in sodium phosphate buffer solution [60]. The SP immobilized on the hydroxyl fullerenes was capable of replacing HRP in H₂O₂ biosensor. In order to construct a SOD mimetic nanocomposite sensor, gold/copper-cysteine (GNPs/Cu-Cys) nanocomposite was synthesized [61]. The effect of GNPs as electron transfer accelerator for O₂^{•-} dismutation, Cys as O₂^{•-} oxidizer, and copper ion as the electron transfer catalyst in O₂^{•-} dismutation, led to the synergic effect of SOD mimetic nanocomposite.

In 2017, a novel method was suggested to identify long nucleotide sequence of human T-lymphotropic virus-1 (HTLV-1) by unmodified cadmium–tellurium quantum dots [3]. The novel designing of this biosensor was such that the single-stranded oligonucleotide was unable to wrap around the quantum dots.

In 2018, an ultrasensitive optical biosensor for microRNA-155 detection was developed in order to diagnose breast cancer at early stages [5]. In this report, the branched positively charged gold nanoparticles (poly-ethylenimine-capped AuNPs) was utilized for the first time to increase loading the target. The proposed biosensor could specify 3-base-pair mismatches and genomic DNA from target miR-155 with the detection limit of 100 aM.

BIOCOMPATIBLE NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

In 2015, silver nanoparticles with antioxidant activity were coated with bovine serum albumin (Ag@BSA) and their antioxidant activity was compared with SOD enzyme [62]. The superoxide radical scavenging assay against

reactive oxygen species suggested that there is a high similarity between the enzymatic activity of Ag@BSA and natural SOD. Also, this nanoparticles showed a strong anti-breast cancer effect.

In 2017 and 2018, several studies were performed to suggest some biocompatible nanoparticles with the chemotherapeutic potential. To this end, the albumin coated cadmium nanoparticles (CdNPs@BSA) was synthesized to be applied as a chemotherapeutic agent against invasive human breast cancer [63]. The resulted CdNPs@BSA could penetrate in tumor micro-vessels and significantly suppressed the breast cancer cells, while they were less toxic on white blood cells. The same activity was observed by albumin coated silver nanoparticles (ASNPs) [64]. The results showed that the median lethal dose (LD₅₀) of ASNPs against breast cancer cells was 30 times higher than that for white normal blood cells. The main mechanism of cell death was detected as apoptosis and reduction of gland tumor sizes. In another work, the novel albumin-based nanocarrier of CuNPs (ACuNPs) was synthesized and their anti-breast cancer and also oxidative stress properties were proved by different methods [65]. The results showed that ACuNPs induces apoptosis in the cancer cells in comparison with CuNPs. Also, the nanoparticle of chitosan/poly(N-isopropylacrylamide-co-acrylic acid)/cellulose laurate [CS/P(NIPAAm-co-AAc/CL)] was synthesized by microfluidic technique and then was used for transdermal multidrug delivery application [66]. The chitosan nanoparticles containing tretinoin and clindamycin phosphate showed a sustained control release of the drugs, minimum inhibitory and antibacterial concentrations compared to the samples synthesized by bulk mixing method.

THE WORKS OTHER THAN BIOELECTROCHEMISTRY AND BIOSENSORS

Pulsed Feld Gel Electrophoresis

To overcome the problems of conventional instruments used in pulsed field and gel electrophoresis such as electrical hazard, electrical noises, formation of gas bubbles at the metal electrodes, a novel electrophoresis unit rely on capacitively-induced pulsed field was designed [67]. The

newly designed electrophoresis unit could resolve the Lambda DNA fragments. The separation of molecules from the wells and the resolution of the bands after increasing the run time were improved compare to the patterns acquired by the conventional electrophoresis.

Spectrophotometric Assay of Horseradish Peroxidase

In 2007, the hydrogen donor couples pyrocatechol-aniline and phenol-aminoantipyrine in the presence of H₂O₂ were compared as chromogens for HRP assay [68]. The pyrocatechol-aniline showed higher sensitivity and lower detection limit relative to those of the phenol-aminoantipyrine couple. So, pyrocatechol-aniline couple was proposed to be a better hydrogen donor for the HRP spectrophotometric assay.

Effects of Mobile Radiation on Hemoglobin

The effects of electromagnetic fields (EMFs) radiation in a specific range on the structure and function of human adult and fetal hemoglobin (HbA and HbF) were studied [69]. For this purpose, the oxygen absorption behaviors of the exposed and unexposed HbA and HbF to EMF were compared. The oxygen absorption value of the exposed HbA was increased while that for HbF was decreased in comparison to those before EMF exposing.

Biofuel Cells

In 2016, two biofuel cells were constructed. Firstly, laccase (Lac) was immobilized in a nanocomposite consisting of PDDA/Lac/PDDA/HOOC-MWCNTs/PMG/Gr which was operated as biocathode [70]. The combination of HOOC-MWCNTs and PDDA led to a suitable microenvironment to establish the activity of immobilized ADH and Lac to facilitate the electron transfer process between redox species (ethanol and oxygen) to the electrodes. Moreover, the electropolymerized layer of PMG on electrodes surfaces could reduce the overpotential of NADH oxidation and oxygen reduction. To develop more novel bioanode for ethanol-oxygen biofuel cell, an enzyme based nanocomposite consisting of polymethylene green as electron transfer mediator, HOOC-MWCNTs as electron transfer accelerator, ADH as biocatalyst and PDDA as supporting agent, β -nicotinamide adenine dinucleotide as

cofactor, and ethanol as fuel was used [71]. The developed biofuel showed the power density of 1.713 mW cm⁻².

In 2017, an efficient microbial fuel cell was constructed [72]. To this end, *Shewanella* algae were grown anaerobically on the woven carbon fiber filament coated with a nanocomposite consisting of NH₂-MWCNTs and a RTIL. *Shewanella* algae could produce the electric current. In addition, an enzyme based fuel cell was developed in which two enzymes were used as bioanode and biocathode. Alcohol dehydrogenase (ADH) was imbedded in a nanocomposite consisting of polydiallyldimethylammonium chloride (PDDA)/ADH/PDDA/HOOC-MWCNTs/polymethylene green (PMG)/GC and used as bioanode.

CONCLUSIONS

Herein, the published results in qualified international journals by the Laboratory of Bio-Analysis during the last decade were overviewed. We represented the new methods for development of different types of nanomaterials to facilitate the signal transduction of biomacromolecules such as proteins and nucleic acids at the surface of physical transducers. Moreover, the application of the developed method for analysis of protein direct electrochemistry, development of biosensors, enzyme/microbial biofuel cells, etc. was introduced.

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