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LABEL-FREE METHODS FOR REAL-TIME ANALYSIS USED IN OXIDATIVE STRESS BIOMARKERS DETECTION

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Abstract: *For human body, oxidative stress is considered pathophysiological mechanism in different pathologies therefore oxidative damage biomarkers detection is real help for clinical purposes. Easy to use devices that can help in the early diagnosis and innovative therapeutic approaches are needed. This paper presents an extensive review of sensitive and specific devices such as biosensors used for real-time detection of oxidative stress biomarkers based on two label-free methods: Electrochemical Impedance Spectroscopy and Surface Plasmon Resonance. Both type of biosensors show very good performances and they are shown to be a viable alternative to conventional methods for oxidative stress biomarkers detection.*

Key words: *oxidative stress, biomarkers detection, EIS, SPR, biosensors.*

1. Introduction

In our days medicine needs easy to use devices that can help in the early diagnosis and innovative therapeutic approaches. The most used methods for bioanalytical detection of diseases biomarkers involve in general sophisticated equipment and protocols. New discoveries and novel technologies are appearing all the time and with the help of molecular diagnostics these are used for clinical purposes. The secreted molecules during inflammation are important in fighting against pathogenic agents. Immune cells produce a series of inflammatory markers such as cytokines and reactive oxygen species (ROS). For human body, oxidative stress is considered pathophysiological mechanism in different pathologies: chronic-inflammatory diseases, cardiovascular diseases, neurological diseases, neoplastic diseases, diabetes, rheumatoid and arthritis [33]. In inflammatory infectious processes in different diseases, such cardiovascular ones, the oxidative damage biomarkers together with immunoglobulin IgM are indicators of oxidative stress [4, 20, 21, 41, 45-47].

Methods presented in literature for detection of ROS are quite complex: direct methods such as electronic spin resonance or indirect methods: chemiluminescence [13, 22], photoluminescence [26] and fluorescence [24] or flow-cytometry of fluorescent marked-H_2O_2 [58]. Enzyme-linked immunosorbent assay (ELISA) to evaluate antibody responses to peptides is used together with immunofluorescence labelling and flow-cytometry, as indirect

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methods using labels for desired biomolecule detection. Thus these methods were used for early diagnosis of Dengue infection by detection of IgM and IgG [55], for detection of IgE, IgG, IgA and IgM against raw and processed food antigens [51]. Evaluation of a rapid assay for detection of IgM antibodies to chikungunya [43] and and IgM detection by indirect and capture ELISAs for the diagnosis of measles and rubella [44] were also reported. Thereby, specific, sensitive and low cost new devices used for biomarkers detection are needed.

This paper presents an extensive review of biosensors used for oxidative stress and acute infection biomarkers detection based on two label-free methods: Electrochemical Impedance Spectroscopy and Surface Plasmon Resonance.

2. Oxidative Stress Biomarkers

ROS is a term used for chemical species obtained after incomplete reduction of oxygen and they are superoxide anion ($O_2^-\bullet$), peroxyl (•OOR), alkoxyl (•OR) and hydroxyl (•OH) as well as nonradical species, hydrogen peroxide (H_2O_2) and are formed in vivo during aerobic metabolism (inner mitochondrial membrane) and in the cellular environment [3, 17]. ROS are the more abundant free radicals in nature, and their pathologic activity for living organisms is caused by an imbalance between their production and destruction. For superoxide anion:

$$O_2 + e^- \rightarrow O_2^-\bullet$$
$$O_2^-\bullet + e^- + 2H^+ \rightarrow H_2O_2$$

For hydroxyl:

$$H_2O_2 + e^- \rightarrow \bullet OH + OH^-$$
$$\bullet OH + OH^- + e^- + 2H^+ \rightarrow 2H_2O$$

Under normal conditions, ROS are neutralized by the action of antioxidants (ROS defence system), and when is overproduction of ROS, an imbalance is created because ROS exceeds the capacity of cellular antioxidant defences to remove toxic oxygen by-products (oxidative stress) [8, 35]. Free radicals generated from mitochondrial respiration, cause oxidative damage of lipids, carbohydrates, proteins and nucleic acids, because free radicals are very unstable and highly reactive species. To make paired electrons, free radicals are searching electrons and they grab them from other molecules. Thus these molecules become unstable and are converted into free toxic radicals. If free toxic radicals are not neutralized or removed, cellular functions will damage, such as: membrane fluidity, membrane transport, enzyme activities and protein functions; therefor cell death finally will produce [12, 48]. Thus, ROS are indicators of inflammatory processes and can serve as markers of interaction between pathogenic agents and immune system [6].

For viral infectious diseases diagnosis, together with clinical symptoms, immunological methods for IgG/IgM detection, molecular methods and viral isolation methods are available but they are either not very specific or they require high-level sophisticated infrastructures.

2.1. Biosensors for Molecular Diagnosis

Using devices that employ biomolecules as analytical tools, offer advantages compared conventional methods due to their simplicity, specificity, selectivity and quick response for real-time analysis of biomolecule interactions.

Biosensors are analytical tools, based on the combination of a biological recognition compound and a physical transducer (sensor). Three basic parts are involved in any biosensor system: biosensing, signal

transduction, and signal readout. The biological recognition element (such as proteins, antibody, DNA or microorganisms) is able to selectively interact with its substrate(s). Biomolecules brings high selectivity and specificity, which are characteristics of biological systems and are very useful for developing of new devices that can be used in medical application [5, 9-11]. It is characteristic that the analytic performance of these devices are obtained by using small quantities of the biologic element, as the functioning of the biosensors is based on the conformational modifications occurring at the level of the biomolecule. In medicine, especially for a rapid screening is ideal to work with very small quantities of biological sample and to detect very small concentrations a target molecule (micromolar or nanomolar). For oxidative stress markers detection few biosensors were developed and majority were electrochemical for H_2O_2 detection. For this oxidases are used as NADPH [2], cytochrome c [56] or horseradish peroxidase [1, 23, 38]. From our knowledge few biosensors were developed for acute infection biomarkers detection, and there are immunoassays-based biosensors when electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis was developed [36] or biosensor based on imaging ellipsometry for detection of hepatitis B virus markers was used [40].

Direct biosensors follow in real time the binding event (following a physical value change) while indirect biosensors measure the result of a binding event, as increasing or decreasing of the label. Direct biosensors do not require labelled species for detection of a target analyte, therefore the detection allows label-free and real-time analysis. Two very sensitive methods can be used for this type of detection for oxidative stress biomarkers: Electrochemical Impedance Spectroscopy and Surface Plasmon Resonance.

2.2. Label-free Impedimetric Biosensors

Electrochemical impedance spectroscopy (EIS) is technique which can be used for the investigation of bulk and interfacial electrical properties of any kind of solid or liquid material which is connected to the appropriate electrochemical transducer [27, 30]. EIS can be used to determine quantitative parameters of electrode processes as well as to provide a fingerprint of its interfacial region. This technique, as SPR also, has the inherent potential for label free detection, which is of special interest in bioanalysis since this circumvents the need to modify biomolecules with fluorescent dyes, enzyme, redox or radioactive labels.

For the impedimetric biosensors the detection principle is based on the changes of the interfacial properties of the electrode in the presence of reversible redox couple using impedance measurements. Electrochemically inert species can be measured by EIS in the presence of a redox agent, such as iron ferrocyanide(s), which undergoes oxidation and reduction at the surface of the electrode, thus making possible label-free detection [31]. EIS follows biosensing events that take place at the surface of an electrode immersed into an electrochemical cell and can be used for detection of the recognition events when immobilized molecules interact with target analytes and to monitor and validate different sensing stages [7]. Immobilized molecule interacts with its target analytes and a complex is formed, an increase of interfacial electron-transfer resistance can be detected by EIS. AC impedance spectra are presented as a Nyquist plot (Z', real impedance, versus –Z", imaginary impedance). The "$-Z_{max}$" value, which corresponds to the highest point of the semicircle on the Nyquist plot is used to characterize the binding. Fig.1 shows the EIS

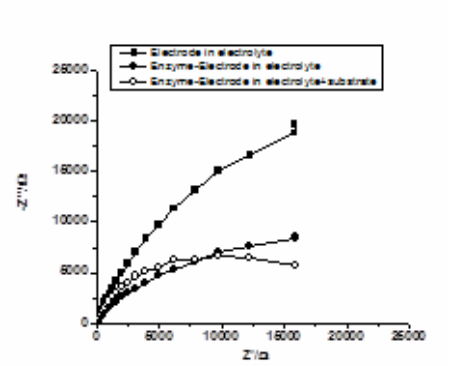

Fig. 1. *Nyquist plot for: bare electrode (■), enzyme-based biosensor in electrolyte without (●) and with (o) enzymatic substrate.*

spectra for glucose-oxidase enzyme-based biosensor in absence and presence of glucose as enzymatic substrate. Finding the maximum values of imaginary part of the impedance in all situations the concentration of the glucose can be obtained directly.

EIS impedance is potentially useful to give insight into cellular behaviour by studying morphological changes, alterations in the physiological state, production of charged or redox species without interfering with in vitro cellular metabolism and labelling [42]. The study has allowed the characterization, from an electrical point of view, of the extracellular NO radical produced endogenously and in great quantities by the inducible form of NO-synthase in the case of LPS-stimulated macrophages. EIS measurements for unstable atherosclerotic plaques that harboured active lipids and inflammatory cells were also performed [57]. Thus it was observed that issue resistance was significantly elevated in the oxidized low density lipoprotein (oxLDL)-rich thin-cap atheromas and fatty streaks as compared with lesion-free region or oxLDL-absent fibrous atheromas.

Biosensor applying aptamer as probe and non-faradic electrochemical impedance spectroscopy (NIS) as the detection method has been developed for monitoring a neuro-inflammatory cytokine PDGF [29]. A good linear relationship between the decrease of capacitance and the logarithm of protein concentration was obtained, which proves the feasibility of quantitative measurements. A sensitive aptamer based electrochemical biosensor to detect human immunoglobulin E (IgE) is presented in reference [39]. Interaction between human IgE and DNA aptamer was EIS monitored in a 0.48nM detection limit of IgE based on electron transfer resistance in the presence of 5mM $[Fe(CN)_6]^{3-/4-}$.

EIS can be used to detect specific interaction antigen-antibody [37]. Thus in literature are described the development of EIS label-free immunosensors for direct detection of three wound infection biomarkers [16] or for detection of h-IgG [60]. Thus, means of unenzymatic-labeling procedure combined with the amperometric detection using dopamine as substrate, the immunological reaction of the immunosensor incubated with h-IgG can be detected. Development of a new sensitive label free impedimetric immunosensor for the detection of MDM2 as prognostic marker for brain tumour is described in reference [19]. The toxins secreted by two clinically important human pathogens, methicillin susceptible Staphylococcus aureus and Pseudomonas aeruginosa were studied via their interaction with a planar tethered bilayer lipid membrane using surface EIS and plasmon resonance spectroscopy [49]. Impedance spectroscopy is also a proven and powerful tool for non-invasive monitoring of cellular processes. Thus it can be proven the hypothesis, that the process of osteogenic differentiation can be monitored non-invasively and time-continuously by using impedance

spectroscopy [25]. A cell-based in vitro exposure system was developed to determine whether oxidative stress plays a role in the cytotoxic effects of volatile organic compounds (VOCs) such as benzene, toluene, xylene, and chlorobenzene, using human epithelial HeLa cells. VOC toxicity was found to correlate with the degree of nitric oxide generation and EIS [15]. The primary reason for the marked increase in impedance was the change of aqueous electrolyte composition as a result of cell responses.

2.2. Label-free Surface Plasmon Resonance biosensors

Surface Plasmon Resonance (SPR) is a surface-sensitive method for bio-analysis as it allows label-free and real-time analysis of biomolecule interactions on functionalized surfaces. The SPR's principle is based on recording of a small change in refractive index of a gold surface modified with molecules with recognizing role. The binding of the target molecules to specific immobilized recognition molecules causes the increasing of surface concentration of target molecules. The sensitivity of the method depends on the extent on which the refractive index is modifying during binding events. This modification will cause a decrease in the intensity of a laser beam when is reflected by modified-gold surface and an increase of its reflexion angle proportional with the concentration of linked target molecules [28, 32].

SPR-based biosensing device for real-time detection of cell differentiation in live cells was developed according to the differences of optical properties of the cell surface caused by specific antigen-antibody binding, without any pre-treatment required in the conventional methods used in molecular biology. SPR biosensor has a strong potential for a high-throughput serodiagnosis of infectious diseases by analysis of mumps virus infection [34] and for acute infection biomarkers detection [18]. Thus an SPR based natural glycan microarray was developed for screening of interactions between glycans and carbohydrate-binding proteins. The SPR screening was sensitive for differences between infection sera and control sera, and revealed antibody titers and antibody classes (IgG or IgM). Results indicate that SPR-based arrays constructed from glycans of natural or synthetic origin, pure or as mixture, can be used for determining serum antibody profiles as possible markers for the infection status of an individual.

Fig. 2 shows the SPR spectra recorded at lectin-based biosensors in the absence and presence of IgM. The lectin Concanavalin A (Con A) binds specifically the receptors containing mannose carbohydrates such as IgM.

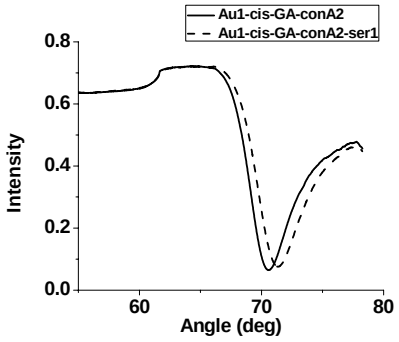

Fig. 2. *SPR spectra of Con A-based biosensor in absence (–) and (--) presence of IgM.*

SPR biosensor was also used to monitor the profiles of the heat-shock protein (DnaK) and the expression of a heterologous protein to map the dynamics of the cellular stress response in Escherichia coli [52]. A novel

nanocomposite $Fe_{(3)}O_{(4)}$-Au nanorod (AuNR) was prepared and used as the substrate in the SPR biosensor to detect goat IgM in the concentration range of 0.15-40.00 μg mL^{-1} [59]. A unique monitoring technique of protein distributions based on distinctive patterns generated by protein adsorption behaviour on a solid surface in a microfluidic channel was described in [14]. Sensing surfaces were pre-adsorbed with one of four different proteins: lysozyme, albumin, transferrin, or IgG and buffer-prepared sample matrices (α1-antitrypsin, haptoglobin, C-reactive protein (CRP), and IgM). Fe_3O_4 nanoparticles-enhanced SPR sensing for ultrasensitive sandwich bio-assay was developed with excellent selectivity for detection of three kinds of proteins (BSA, human IgM and human IgE) [54]. SPR immunosensor based on core/shell Fe_3O_4/Au nanocomposites developed in [53] exhibits a satisfactory response for human IgM in the concentration range of 0.30-20.00 μg mL^{-1}. SPR spectroscopy was used to monitor the presence of serum antibodies to neoglycoconjugates containing defined carbohydrate epitopes and to define the IgM and IgG subclass distribution of the antibodies [50].

3. Conclusions

Thereby, the literature describes both label-free methods for many types of biomarkers detection. We can conclude, however, that few biomarkers for oxidative damage were detected using EIS-based biosensors and even few for biomarkers of acute infection. Also, SPR-biosensors are rarely used to detect oxidative stress biomarkers and they are used for detection of antibodies as acute infection markers. The concentrations of detected markers were very low (micromolar and nanomolar) which they recommend these methods to be used for developing sensitive and selective biosensors as alternatives of conventional methods.

References

1. Ahammad, AJ, Sarker, S, Lee, J.J.: *Immobilization of horseradish peroxidase onto a gold-nanoparticle-adsorbed poly(thionine) film for the construction of a hydrogen peroxide biosensor*. In: J Nanosci Nanotechnol (2011) Vol. 11(7), p. 5670-5.
2. Ashkenazi, A., Abu-Rabeah K., Marks R.S.: *Electrochemistry and chemiluminescence techniques compared in the detection of NADPH oxidase activity in phagocyte cells*. In: Talanta (2009) Vol. 77(4), p. 1460-1465.
3. Bartosz, G.: *Reactive oxygen species: Destroyers or messengers.* In: Biochemical Pharmacology (2009) Vol. 77, p. 1303-1315.
4. Bendall, R., Ellis, V., Ijaz, S., et al.: *Serological response to hepatitis E virus genotype 3 infection: Igg quantitation, avidity, and Igm response*. In: J Med Virol. (2008) Vol. 80(1), p. 95-101.
5. Bilitewski, U., Jager, A., Ruger, P., Weise, W.: *Enzyme Electrodes for the Determination of Carbohydrates in Food*. In: Sensors and Actuators B (1993) Vol. 113, p. 15-16.
6. Yan, J., Pedrosa, V. A., Enomoto, J., et al.: *Electrochemical Biosensors for On-Chip Detection of Oxidative Stress from Immune Cells*. In: Biomicrofluidics (2011) Vol. 5(3), p. 32008-32011.
7. Brett, C. M. A.: *Electrochemical Impedance Spectroscopy for Characterization of Electrochemical Sensors and Biosensors*. In: ECS Transactions (2008) Vol. 13(13), p. 67-80.

8. Brigelius-Flohe, R.: *Vitamin E. The shrew waiting to be tamed*. In: Free Radical Biology and Medicine (2009) Vol. 46, p: 543-554.
9. Bruckenstein, S., Shay, M.: *An in Situ weighing study of the mechanism for the formation of the adsorbed oxygen monolayer at a gold electrode*. In: J. Electroanal. Chem. Interfacial Electrochem. (1985) Vol. 188, p. 131-135.
10. Buttry, D.A., Ward, M.D.: *Measurement of Interfacial Processes at Electrode Surfaces with the Electrochemical Quartz Crystal Microbalance*. In: Chem. Rev. (1992) Vol. 92, p. 1335-1341.
11. Canh, T.M.: *Biosensors*. London. Chapman & Hall, 1993.
12. Chauhan, V., Chauhan, A.: *Oxidative stress in Alzheimer's disease*. In: Pathophysiology (2006) Vol. 13, p. 195-208.
13. Chen, W.W., Li, B.X., Xu, C.L., Wang, L.: *Chemiluminescence flow biosensor for hydrogen peroxide using dnazyme immobilized on eggshell membrane as a thermally stable biocatalyst*. In: Biosen. Bioelectron. (2009) Vol. 24, p. 2534-2539.
14. Choi, S., Huang, S., Li, J., Chae, J.: *Monitoring protein distributions based on patterns generated by protein adsorption behavior in a microfluidic channel*. In: Lab Chip. (2011) Vol. 11(21), p. 3681-3688.
15. Chung, S.G., Kim, J.C., Park, C.H., et al.: *Volatile organic compound specific detection by electrochemical signals using a cell-based sensor*. In: J Microbiol Biotechnol. (2008) Vol. 18(1), p. 145-152.
16. Ciani, I., et al.: *Development of immunosensors for direct detection of three wound infection biomarkers at point of care using electrochemical impedance spectroscopy*. In: Biosensors and Bioelectronics (2012) Vol. 31, p. 413– 418.
17. Circu, M., Aw, T.Y.: *Reactive oxygen species, cellular redox systems, and apoptosis.* In*:* Free Radical Biology and Medicine (2010) Vol. 48, p. 749-762.
18. de Boer, A.R., Hokke, C.H., Deelder, A.M.: *Serum antibody screening by surface plasmon resonance using a natural glycan microarray*. In: Glycoconj J. (2008) Vol. 25(1), p. 75-84.
19. Elshafey, R., Tlili, C., Abulrob, A.: *Label-Free impedimetric immunosensor for ultrasensitive detection of cancer marker murine double minute 2 in brain tissue*. In: Biosens Bioelectron. (2013) Vol. 39(1), p. 220-225.
20. Gutteridge, J.M.C., Mitchell, J.: *Redox Imbalance in the critically ill*. In: Br. Med. Bull. (1999) Vol. 55, p. 49-56.
21. Haliwell, B., Gutteridge, J.M.C.: *Lipid peroxidation, O_2, radicals, cell damage and antioxidant therapy*. In: Lancet (1984) Vol. 1, p. 1396-1402.
22. Han, L., Zhang, Y., Kang, J., Tang, J., Zhang, Y.: *Determination Co^{2+} in Vitamin B12 based on enhancement of 2-(4-Substituted-Phenyl)-4,5-Di(2-Furyl) Imidazole and H_2O_2 chemiluminescence reaction*. In: Spectrochim Acta A Mol Biomol Spectrosc. (2011) Vol. 82(1), p. 146-152.
23. Han, M., Liu, S., Bao, J., Dai, Z*.: Pd nanoparticle assemblies--As the substitute of HRP, in their biosensing aspplications for H_2O_2 and glucose*. In: Biosens. Bioelectron. (2012) Vol. 31(1), p. 151-156.
24. Heller, D. A. et al.: *Multimodal optical sensing and analyte specificity using single-walled carbon nanotubes*. In: Nature Nanotech. (2009) Vol. 4, p. 114–120.
25. Hildebrandt, C., Büth, H., Cho, S., et al.: *Detection of the osteogenic differentiation of mesenchymal stem cells in 2D and 3D cultures by electrochemical impedance spectroscopy*. In: J Biotechnol. (2010) Vol. 148(1), p. 83-90.

26. Jin, H., Heller, D.A., Kalbacova, M. et al.: *Detection of single-molecule H_2O_2 signalling from epidermal growth factor receptor using fluorescent single-walled carbon nanotubes*. In: Nat. Nanotechnol. (2010) Vol. 5, p. 302-308.
27. Komarova, E. K., Reber, Aldissi, M., Bogomolova, A.: *New Multispecific Array As a Tool for Electrochemical Impedance Spectroscopy-Based Biosensing. In:* Biosensors and Bioelectronics (2010) Vol. 25, p. 1389–1394.
28. Kuo, Y.C., Ho, J.H., Yen, T.J., et al.: *Development of a surface plasmon resonance biosensor for real-time detection of osteogenic differentiation in live mesenchymal stem cells.* In: PLoS One. 2011 Vol. 6(7), p.e22382.
29. Liao, W., Cui, X.T.: *Reagentless aptamer based impedance biosensor for monitoring a neuro-inflammatory cytokine PDGF*. In: Biosens Bioelectron. (2007) Vol. 23(2), p. 218-224.
30. Lisdat, F., Schäfer, D.: *The use of electrochemical impedance spectroscopy for biosensing*. In: Anal. Bioanal. Chem. (2008) Vol. 391, p. 1555-1567.
31. Loo, A.H., Bonanni, A., Pumera, M.: *Impedimetric thrombin aptasensor based on chemically modified graphenes*. In: Nanoscale (2012) Vol. 4, p. 143-147.
32. Lundstrom, I.: *Real-Time Biospecific Interaction Analysis*. In: Biosens. Bioelectron (1994) Vol. 9, p. 725-736.
33. Mena, S., Ortega, A., Estrela, J.: *Oxidative Stress in Environmental-Induced Carcinogenesis*. Mutation Research, (2009) Vol. 674, p.36-44.
34. Yuk, J.S., Ha, K.S: *Array-Based Spectral SPR Biosensor: Analysis of Mumps Virus Infection*. In: Methods Mol Biol. (2009) Vol. 503, p. 37-47.
35. Pacheco, J.L., Gonesebatt, M.: *The Role of Antioxidants and Antioxidant-Related Enzymes in Protective Responses to Environmentally Induced Oxidative Stress*. In: Mutation research, (2009) Vol. 674, p. 137-147.
36. Pan, Y., et al.: *Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis*. In: Biosens Bioelectron. (2010) Vol. 26(2), p. 649-655.
37. Patolsky, F. Filanovsky, B. Katz, E. Willner, I.: *Photoswitchable Antigen-antibody interactions studied by impedance spectroscopy*. In: J. Phys. Chem. B (1998) Vol. 102, p. 10359-10367.
38. Peng, Y., Jiang, Su, L., Zhang, D.L., et al.: *Mixed Monolayers of ferrocenylalkanethiol and encapsulated horseradish peroxidase for sensitive and durable electrochemical detection of hydrogen peroxide*. In: Anal. Chem. (2009) Vol. 81, p. 9985-9991.
39. Kara, P., Meric, B., Ozsoz, M.: *Development of a label free IGE sensitive aptasensor based on electrochemical impedance spectrometry*. In: Combinatorial Chemistry & High Throughput Screening (2010), Vol. 13(7), p. 578-581.
40. Qi, C., et al.: *Detection of Hepatitis B virus markers using a biosensor based on imaging ellipsometry*. In: J Viral Hepat. (2009) Vol. 16(11), p. 822-832.
41. Ramsland, P.A., et al.: *Structural basis for Fc Gammariia recognition of human Igg and formation of inflammatory signaling complexes*. In: J Immunol. (2011) Vol. 187(6), p. 3208-3217.
42. Reybier, K, Ribaut, C, Coste, A.: *Characterization of oxidative stress in leishmaniasis-infected or LPS-stimulated macrophages using electrochemical impedance spectroscopy*. In: Biosens Bioelectron. (2010) Vol. 25(12), p. 2566-2572.

43. Rianthavorn, P. et al.: *Evaluation of a rapid assay for detection of Igm antibodies to Chikungunya*. In: Southeast Asian J Trop Med Public Health. (2010) Vol. 41(1), p. 92-96.
44. Sanz, J.C. et al.: *Assessment of RNA amplification by multiplex RT-PCR and IgM detection by indirect and capture Elisas for the diagnosis of Measles and Rubella*. In: APMIS. (2010) Vol. 118(3), p. 203-209.
45. Selim, H.S., Abou-Donia, H.A., Taha, H.A., et al.: *Role of occult hepatitis B virus in chronic Hepatitis C patients with fare of liver enzymes*. In: Eur J Intern Med. (2011) Vol. 22(2), p. 187-190.
46. Seriwatana, J, Shrestha, MP, Scott, RM.: *Clinical and epidemiological relevance of quantitating Hepatitis E virus-specific immunoglobulin M*. In: Clin Diagn Lab Immunol. (2002) Vol. 9(5), p. 1072-1078.
47. Shoujie H., et al.: *Profile of acute infectious markers in sporadic Hepatitis E.* In: PLoS One. (2010) Vol. 5(10), p. e13560- e13566
48. Trushina, E., McMurray, C.*: Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases*. In: Neuroscience (2007) Vol. 145, p. 1233-1248.
49. Tun, T.N., Cameron, P.J., Jenkins, A.T.: *Sensing of pathogenic bacteria based on their interaction with supported bilayer membranes studied by impedance spectroscopy and surface plasmon resonance*. In: Biosens Bioelectron. (2011) Vol. 28(1), p. 227-231.
50. van Remoortere, A., van Dam, G.J., Hokke, C.H., et al.: *Profiles of immunoglobulin M (Igm) and Igg antibodies against defined carbohydrate epitopes in sera of Schistosoma-infected individuals determined by Surface Plasmon Resonance*. In: Infect Immun. (2001) Vol. 69(4), p. 2396-401.
51. Vojdani, A.: *Detection of IgE, IgG, IgA and IgM antibodies against raw and processed food antigens*. In: Nutr Metab (Lond). (2009) Vol. 12, p. 6-22.
52. Vostiar, I., Tkac, J., Mandenius, C.F.: *Off-Line monitoring of bacterial stress response during recombinant protein production using an optical biosensor*. In: J. Biotechnol. (2004) Vol. 111(2), p. 191-201.
53. Wang, J., Sun, Y., Wang, L.: *Surface plasmon resonance biosensor based on Fe_3O_4/Au nanocomposites*. In: Colloids Surf B Biointerfaces. (2010) Vol. 81(2), p. 600-606.
54. Wang, J., Zhu, Z., Munir, A., Zhou, H.S.: *Fe_3O_4 Nanoparticles-enhanced SPR sensing for ultrasensitive sandwich bio-assay. In:* Talanta (2011) Vol. 84(3), p. 783-788.
55. Wang, S.M, Sekaran, S.D.: *Early diagnosis of dengue infection using a commercial dengue duo rapid test kit for the detection of NS1, IGM, and IGG*. In: Am J Trop Med Hyg. (2010) Vol. 83(3), p. 690-695.
56. Yagati, A.K., Lee, T., Min, J., Choi, J.W.: *Electrochemical Performance of Gold Nanoparticle-Cytochrome C Hybrid Interface for H_2O_2 Detection*. In: Colloids Surf B Biointerfaces. (2012) Vol. 92, p. 161-167.
57. Yu, F., Dai, X., Beebe, T., Hsiai, T.: *Electrochemical impedance spectroscopy to characterize inflammatory atherosclerotic plaques*. In: Biosens Bioelectron. (2011) Vol. 30(1), p. 165-173.
58. Zawada, W.M., et al.: *Generation of reactive oxygen species in 1-Methyl-4-Phenylpyridinium (MPP+) treated dopaminergic neurons occurs as an NADPH Oxidase-dependent Two-wave Cascade*. In: J Neuroinflammation. (2011) Vol. 8, p. 129-135.

59. Zhang, H., Sun, Y., Wang, J.: *Preparation and application of novel nanocomposites of magnetic-Au nanorod in SPR Biosensor*. In: Biosens Bioelectron. (2012) Vol. 34(1), p. 137-143.

60. Zhang, L., Liu, Y., Chen, T.: *A mediatorless and Label-Free amperometric immunosensor for detection of H-Igg*. In: International Journal of Biological Macromolecules (2008) vol. 43, p. 165–169.