Innovative Immunosensors for Early Stage Cancer Diagnosis and Therapy Monitoring

Cecilia Cristea[1], Anca Florea[1], Ramona Galatus[2], Ede Bodoki[1], Robert Sandulescu[1], Daniel Moga[2], and Dorin Petreus[2]

[1]"Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Pharmacy, Analytical Chemistry Department, Cluj-Napoca, Romania
[2]Technical University of Cluj-Napoca, Cluj-Napoca, Romania

Abstract— **This paper addresses the development of a diagnosis device based on optical and electrochemical principles for the detecting the levels of multiple mucin (MUC) type biomarkers. Combining surface plasmon resonance (SPR) and electrochemical techniques the selectivity can lead to improved sensitivity and reliability of the obtained transducer. The structures appropriate for an easy operated device, not requiring highly skilled operating personnel or expensive instrumentation, while offering fast, selective and accurate analytical response are investigated. A diagnosis device based on such combined immunosensor can greatly improve the chances of early diagnosis of ovarian cancer.**

Keywords—**electrochemical immunosensor, SPR phenomenon, antibody, aptamer, gold nanoparticles**

I. INTRODUCTION

Recently, immunology and cancer research has identified several molecules, including the mucin (MUC) family of glycoproteins, exploitable as biomarkers for breast and ovarian cancers. Because cancer of the ovary confers the worst prognosis when malignancies are diagnosed at late stage, an early stage diagnosis based on the monitoring of multiple biomarkers could significantly contribute to the evolution and prognosis of these types of cancer. The determination of serum tumor markers plays an important role in the screening and diagnosis.

Mucins are large glycoproteins with important role in the protection and lubrication of the surface of epithelial tissues including mammary gland, female reproductive tract, lung, kidney, stomach, gall bladder or pancreas, as well as in cell differentiation, signaling processes and cell adhesion [1]. Recent studies revealed the implication of some mucins (such as MUC1, MUC4, MUC16) in the pathogenesis of ovarian and breast cancer [2-4]. Under normal physiological conditions the production of mucins is maintained in optimum range. In the case of a neoplastic process the proteins are overexpressed and enter into blood circulation where they can be measured by different assays, serving therefore as tumor markers.

The monitoring of a single MUC type molecule does not allow precise cancer diagnosis. However, correlating the levels of multiple MUC type biomarkers determined by the means of high throughput and low cost sensor arrays with the aid of multivariate data analysis increases the number of correctly diagnosed patients in the early stages of cancer.
One of the most widely used entities for biomarker detection is the antibody molecule, which provides the specificity and sensitivity required for low levels of molecular detection. Recently, synthetic (artificial) molecular recognition elements have been fabricated as affinity materials and used for analyte detection and analysis: nanomaterials and membrane structures which can comprise molecular imprinted polymers, aptamers, and phage display peptides, binding proteins and synthetic peptides as well as metal oxides [5]

Immunosensors can be defined as affinity ligand-based biosensing devices, which combine the specificity of the affinity immunoreaction between an antibody and its specific antigen with the sensitivity of different physical transducers [6]. Electrochemical immunosensors possess several advantages over more conventional techniques (enzyme-linked immunosorbent assay, fluoroimmunoassay, radioimmunoassay), being less time- and reagent-consuming, and having low cost. [7].

Lately, many sensors used as transducers screen printed electrodes (SPEs) having as working electrodes the graphite or gold based electrode. Their surface is easily modifiable with gold nanoparticles (AuNPs), thin films or carbon nanotubes.

If the surface plasmon resonance technique (SPR) is combined with spectroelectrochemical and impedimetric-methods, a new sensor could be devised for selective biomarkers' detection without passing through several incubation steps and without using different types of antibodies. The development of a diagnosis device based on optical and electrochemical principles, without requiring highly skilled operating personnel and expensive instrumentation, as well

I. Lacković et al. (eds.), *The International Conference on Health Informatics*, IFMBE Proceedings 42,
DOI: 10.1007/978-3-319-03005-0_13,

as fast, selective and accurate analytical response will greatly improve the chances of early diagnosis and improved therapeutic outcome.

To excite surface plasmons, generally, a prism is used (Kretschmann configuration), but the low cost solution investigated in this paper is based on a D-type step-index multimode POF (plastic optical fiber.

Some assays related to mucines were already developed [7-11]. The final purpose of our study will be related with the design of a multiarray of hybrid sensors (optical and electrochemical simultaneous detection) for several cancer biomarkers related to ovarian and breast cancer.

II. METHODS

A. SPR Sensor

The fiber used in the construction of the sensor is no longer cylindrically symmetrical after side-polishing, and due to shape of its cross-section is called a D-type fiber. The optimum buffer layer (Microposit 1813 photoresistor) and its deposition techniques were investigated in order to achieve the optimum sensitivity and good adhesion of the subsequent gold layer onto a D-surface.

The planar gold layer will also allow an easy-binding of the antibody to the sensor's surface. The state of linearly polarized light oriented parallel to a polished surface is the TE mode. Its orthogonal state is the TM mode that has contribution to the plasmonic effect (*p*-wave). The transfer matrix formalism (TMF) simulation was done to obtain the resonance wavelength of the POF-SPR sensor [12]. Following the intensity method detection, the setup consists of a broad-band halogen lamp source, with λ=400~1300nm with narrow spot (Ocean Optics: Model no. HL-2000-LL), SPR sensor and a spectrum analyzer, with a detection range from 200 nm to 850 nm (Ocean Optics "USB2000+UV-VIS", FWHM=1.5nm).

B. Imunosensor development

Typically, in the development of an immunosensor several steps are involved. The first step consists in the modification of the electrode's surface with gold nanoparticles, carbon nanotubes, graphenes, nanofibers, conducting polymers, in order to increase the conductivity and obtain higher sensitivities; this structures also constitute immobilization platforms for the bioreceptors. The second step is in fact the immobilization of the bioreceptor (aptamer, antibody) onto the surface of the modified electrode. The affinity reaction between the immobilized bioreceptor and the protein takes place in the next step. In the last step the reaction of the antibody-antigen- or aptamer-antigen-complex with a secondary antibody or aptamer, labeled with either an enzyme or an electroactive compound is taking place in order to obtain a measurable electrochemical signal.

All the experimental conditions involved in each step mentioned above were carefully optimized through electrochemical methods, in order to obtain the best performance of the sensor [8]. Factors such as pH, buffers, temperature, concentration of nanostructures, monomers and incubation times with aptamers, antibodies, tumor markers were investigated.

III. RESULTS AND DISCUSSIONS

A. SPR sensor

The most common commercially available POF material is poly-methyl metacrylate (PMMA), long known for being non-toxic and showing good biological compatibility. The fiber has locally removed cladding along half circumference, the section with exposed core being approximately 10 mm long. The easily processed plastic material permits a controllable side-polishing procedure.

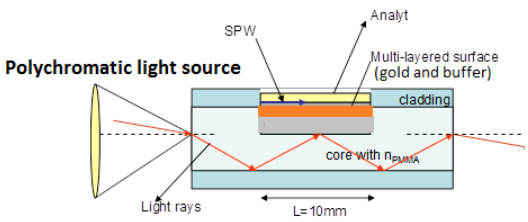


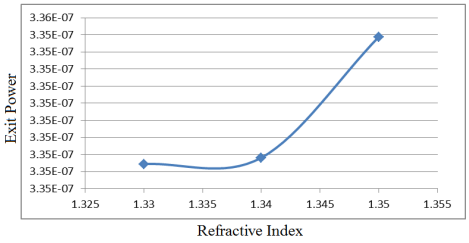
Figure 1. SPR sensor, longitudinal section

PMMA-based POFs are suitable for light transmission in the visible and near infrared range (450nm-850 nm). The lowest POF attenuation in the 520 nm and 650 nm bands are below 0.2 dB/m, allowing the fiber to carry signals to remote opto-electronic device if it is necessary.

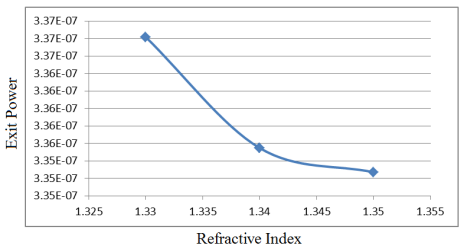
Because of the fixed geometry of the fiber sensor (figure 1), the preferred mode of SPW-surface plasmon wave (and hence the marker) detection is based on spectral analysis of insertion loss experienced in the fiber device. SPW results in a peak of insertion loss at certain wavelength (identifying type of the marker) and amplitude (indicating its concentration).

The FDTD simulation was done to determine the variation of the exit power with the refractive index of the analyte. The resulting structure with a buffer layer of 1.5 μm and gold layer of 40 nm provide a good sensor like behavior (figure 2), in good agreement with previously published results [13]. Experimentally obtained SPR transmission spectra for different refractive index values of the

sample medium, normalized with the air spectrum (figure 3) showed a good agreement with the TMF simulation method.



a.



b.

Figure. 2 Simulation results, variation of the exit power: a) using 500nm wavelenght, b) using 650 nm wavelenght

The shift in resonance wavelength in the range of interest, per unit change in the refractive index of the sensing region is of the order of 1 x10^3[nm/RIU]).

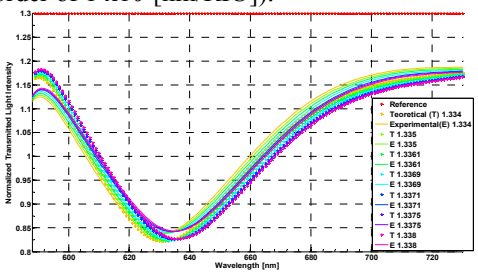


Figure 3. SPR transmission spectra

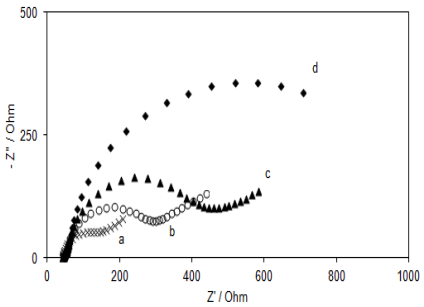
B. MUC 1 Immunosensor

Our approach involved the modification of the gold screen printed electrodes by electrodeposition of gold nanoparticles (AuNPs) [8]. The immobilization of the nanoparticles on the electrode increases the surface area of the electrode and promotes the adsorption capability of the electrode.

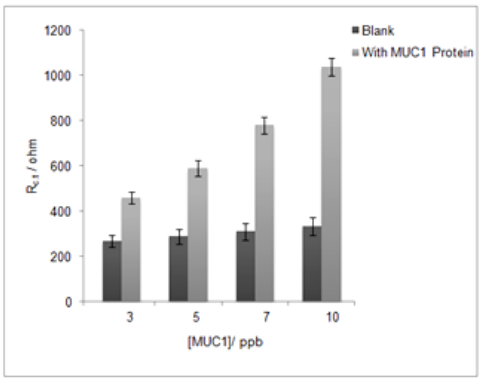
The gold surface of SPEs was cleaned by cyclic voltammetry (CV) in sulfuric acid 0.5M. After this step gold nanoparticles were electrodeposited on the surface of gold SPEs by CV from a solution of 0.6mM $HAuCl_4$ in H_2SO_4 0.5M by cycling the potential in the range of -0.2 to 1.2V vs.Ag/AgCl at a scan rate of 100mVs^{-1} for 15 cycles. A key step in the production of AuNPs was the optimization of the number of cycles for electrodeposition (5,10,15 cycles) the dimensions and the number of the AuNP affecting the analytical performances of the immunosensors.

The next step in the development of the electrochemical aptasensors is the immobilization of the aptamer. First the incubation of the gold nanoparticles modified electrode with 10µM of thiolated aptamer overnight was performed. An optimization of the incubation time with the aptamer (2h, overnight) was necessary.

Another step in the construction of the aptasensor was blocking the nonspecific sites with 1mM 6-mercaptohexanol (MCH) for 1 hour. A supplementary optimization step of the incubation time with MCH was necessary in order to assess the adequate time.



A.



B.

Figure 4. (A.) Nyquist plot of the immunosensor in 10 mM $[Fe(CN)_6]^{-3/-4}$ solution for: bare gold SPE (a), gold SPE/AuNPs/Aptamer (b), gold SPE/AuNPs/Aptamer/MUC1 protein (3ppb) (c), gold SPE/AuNPs/Aptamer/ MUC1 protein (10ppb) (d). (B.) Change of the electron-transfer resistances (R_{ct}) at the aptamer-AuNPs modified gold electrodes upon the analysis of different concentration of MUC1 protein (0, 3, 5, 7,10 ppb).

The gold SPEs were modified with AuNPs as immobilization platform for the thiolated aptamers and the features of the sensor were investigated by electrochemical impedance spectroscopy (EIS), using 10 mM $[Fe(CN)_6]^{-3/-4}$ in PBS

pH=7.4 as a redox probe. A decrease in the charge transfer resistance was observed after electrodeposition of AuNPs (a) with respect to the gold electrode (b), improving the electron transfer at the electrode surface as shown in Figure 4A.

The immobilization of the aptamer on the AuNPs modified surface increased the electron transfer resistance (R_{ct}) (c). After incubation with MUC1 protein (different concentration 3-10 ppb), the interfacial resistance increased more due to the interaction of aptamer with MUC1 protein (d). The relative change in R_{ct} has been chosen as signaling parameter. As the concentration of the MUC1 protein increases, the resultant electron transfer resistance was enhanced. A dose-response curve was obtained under optimized experimental conditions by EIS technique (Fig 4B). A linear response was obtained in the range of 0-10 ng mL^{-1} (R_{ct} = 292.02+ 67.155x , R^2 = 0.9831) with a limit of detection of 1.66 ng mL^{-1}.

IV. CONCLUSIONS

The major goal of the study is the development of new, non-invasive devices for early diagnosis of ovarian cancer and for the therapy monitoring which are more specific and sensitive with respect to the currently used methods. After the successful development of a sensor for each tumor marker, a multiarray for simultaneous detection of several markers will be taken in consideration for more accurate results. The proposed hybrid sensor combines the plasmonic sensor with a three electrode electrochemical cell (working, counter and reference electrodes) where the gold layer responsible for the plasmonic effect, can serve as working electrode for electrochemical and impedimetric investigations.

ACKNOWLEDGMENT

The authors acknowledge Prof. Giovanna Marrazza and Zahra Taleat from University of Firenze, Italy for the helpful discussions. The authors are grateful for the financial support to the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0355.

REFERENCES

1. M. Andrianifahanana, N. Moniaux, SK Batra, Regulation of mucin expression: Mechanistic aspects and implications for cancer and inflammatory diseases, Biochim Biophys Acta. 2006,1765,189–222
2. L. Wang, J. Ma, F.H. Liu, Q.K. Yu, G.M Chu, et al, Expression of MUC1 in primary and metastatic human epithelial ovarian cancer and its therapeutic significance, Gynecol Oncol. 2007, 105(3), 695-702
3. B. Davidson, M. Baekelandt, I.M. Shih. MUC4 is upregulated in ovarian carcinoma effusions and differentiates carcinoma cells from mesothelial cells. Diagn Cytopathol. 2007 , 35(12), 756-60.
4. C.Thériault, M. Pinard, M. Comamala, M. Migneault, J. Beaudin et al. MUC16 (CA125) regulates epithelial ovarian cancer cell growth, tumorigenesis and metastasis, Gynecol Oncol. 2011, 121(3), 434-443
5. I. Diaconu, C. Cristea, V. Hârceagă, G. Marrazza, I. Berindan-Neagoe, R. Săndulescu, Electrochemical immunosensors in breast and ovarian cancer, Clinica Chimica Acta, Vol 425, 128-138
6. N. Bojorge Ramirez, A.M. Salgado, B. Valdman, The evolution and developments of immunosensors for health and environmental monitoring: problems and persepectives, Brazilian journal of chemical engineering, 2009, 26(2), 227-249
7. M. Hasanzadeh, N. Shadjou, M. Eskandani, M. de la Guardia, E. Omidinia, Electrochemical nano-immunosensing of effective cardiac biomarkers for acute myocardial infarction, TrAC Trends in Analytical Chemistry, 2013, 49, 20-30
8. A. Florea, Z. Taleat, C. Cristea, M. Mazloum-Ardakani, R. Săndulescu, Label free MUC1 aptasensors based on electrodeposition of gold nanoparticles on screen printed electrodes, Electrochemistry Communications, 2013, 33, 127-130
9. J. Zhao, X. He, B. Bo, X. Liu, Y. Yin, G. Li, A "signal-on" electrochemical aptasensor for simultaneous detection of two tumor markers, Biosensors and Bioelectronics, 2012, 34, 249-252
10. A. Ravalli, G. Pilon dos Santos, M. Ferroni, G. Faglia, H. Yamanaka, G. Marrazza, New label free CA125 detection based on gold nanostructured screen-printed electrode, Sensors and Actuators B: Chemical, 2013, 179, 194-200
11. W. Liu, Y. Zhang, S. Ge, X. Song, J. Huang, M. Yan, J. Yu, Core–shell Fe_3O_4–Au magnetic nanoparticles based nonenzymatic ultrasensitive electrochemiluminescence immunosensor using quantum dots functionalized graphene sheet as labels, Analytica Chimica Acta, 2013, 770, 132-139
12. G. Wang , RJ Lipert, M Jain, S Kaur, S Chakraboty, et al. Detection of the Potential Pancreatic Cancer Marker MUC4 in Serum Using Surface-Enhanced Raman Scattering, *Anal. Chem.*, 2011, 83 (7), 2554–2561
13. Nunzio Cennamo, Davide Massarotti, Laura Conte, Luigi Zeni, Low Cost Sensors Based on SPR in Plastic Optical Fiber for Biosensor Implementation, Sensors 2011, 11(12), 11752-11760, http://www.mdpi.com/1424-8220/11/12/11752 ,
14. R. Galatus, N. Cennamo, E. Voiculescu, Optimal Design of D-type Plastic Fibers for best sensitivity of SPR sensors, Advanced Engineering Forum 2013, 8-9, 563-573. http://www.scientific.net/AEF.8-9.563

Corresponding author:

Author: Daniel Moga
Institute: Technical University of Cluj-Napoca, Faculty of Automation and Computer Science, Automation Department
Street: 15, C.Daicoviciu St., 400020
City: Cluj-Napoca
Country: Romania
Email: daniel.moga@aut.utcluj.ro