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Optical Biosensing Analysis of Spermine as Marker of Prostate Cancer with Help of Colloidal Gold and Anti-Spermine Antibody Modified Transducers

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ABSTRACT

Prostate cancer (PC) is a deadly oncological disease spread all around the world among elderly men. Existing methods of analysis or detection of traditional biomarkers has proven to be expensive and in some cases less precise. That is why in recent years polyamines are studying as potential PC biomarkers. On the other hand biosensors have already proven themselves as precise and sensitive methods that can be used diagnostics, namely in detection of polyamines. Therefore, the aim of this research was to develop a new type of transducer for SPR biosensor based on use of colloidal gold and compare it with conventional antibody-antigen transducer to detect spermine as prostate cancer biomarker. The analysis was made with help of two biosensing devices Plasmontest and SPR-6 which were used detect spermine in range from 0.5 to 1000 nM During the research it was investigated that transducer treated with colloidal have higher sensivity to spermine comparing with antibody-treated transducer. It was observed that range of detected spermine concentration varies from 0.5 to 1000 nM and oplimum located in range 10-500 nM. This article presents data related to the development of the modern sensitive biosensor capable of detecting spermine as a potential marker of prostate cancer.

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Introduction

Prostate cancer (PC) is one of the most common malignancies in men over the age of 50. In most countries of Europe and America, PC ranks first in the structure of cancer among men. The rate of increase in the incidence of PC is one of the highest among all malignant neoplasms [1, 2]. A promising direction in the diagnostics of polyamines, in particular spermine, is the use of biophysical diagnostic methods, such as Raman spectroscopy, photoluminescence analysis, spectroscopy using localized plasmon resonance, liquid or gas mass spectrometry, as well as biosensors, in particular optical [3]. Each of them has its advantages and disadvantages, but these methods, compared with the existing ones, are used in the diagnosis of prostate cancer: determining the level of prostatic specific antigen (PSA) in the blood, prostate biopsy, transrectal echography, magnetic resonance imaging, etc., which are either expensive, time-consuming, or involve surgical or any other physical intervention in the patient's body. The most effective is the use of optical biosensors, in particular due to the ease of use and high specificity due to the use of monoclonal antibodies as well as other molecules such as gold nanoparticles against spermine and other polyamines. In addition, gold nanoparticles have the ability to form self-assembling layers on any surface, in particular on a glass substrate, or on a substrate covered with a layer of gold, which makes it possible to use layers of gold nanoparticles, in particular colloidal, stabilized with sodium citrate, for biosensors based on the phenomenon of surface plasmon resonance (SPR) for the diagnosis of prostate cancer [4]. The variety of available nanomaterials opens the door to the development of new strategies for detecting biomarkers of PC. Gold nanoparticles exhibit attractive optical properties due to their interaction with light, in addition to high electrical conductivity and resistance to oxidation [5, 6]. Due to their internal optical, electronic and physicochemical properties, Au nanoparticles are widely investigated for use in biomedicine [7]. In recent years, appropriate progress has been made in the use of Au nanostructures for clinical and preclinical diagnosis.

Materials and Methods

Studies of the interaction of AuNPs with spermine as a marker of prostate cancer using the phenomenon of SPR on biosensor devices "Plasmontest" and "SPR-6" have been made. Colloidal gold, synthesized and kindly provided by the staff of the Chuiko

Institute of Surface Chemistry of the NAS of Ukraine, was obtained and stabilized using sodium citrate. The concentration of metallic Au in the colloidal solution was $3*10^{-4}$M or 60 mg/ml. The sizes of Au nanoparticles, determined by the method of diffuse light scattering (DLS), were in the range of 13-25 nm with help of laser correlation spectrometer Zeta Sizer Nano S equipped with a correlator (Multi Computing Correlator Type 7032 CE. In order to radiumize a suspension the helium-neon laser LGN–111 was used. Scattering light had been registered and processed statistically at 173 degrees for 120 seconds at 25 °C. The autocorrelation was performed with standard software PCS–Size mode v.1.61 [8]. Methods of biosensor analysis with the use of optical biosensor devices SPR-6 and Plasmontest, kindly provided by V. E. Lashkaryov Institute of Semiconductor Physics NAS of Ukraine and V. M. Glushkov Institute of Cybernetics of National Academy of Sciences of Ukraine. A comparative analysis of substrates modified with a layer of colloidal gold and antibodies was carried out. A substrate with a sprayed layer of gold or antibodies against spermine (Abcam, USA) was pre-treated with a layer of polyelectrolytes, namely polyallylamine (PAA) to increase the sensitive surface of the developed biosensor. Later, when measurements were started, distilled water (H_2O) was used to wash measurement chamber. Then a solution of stabilized colloidal gold was introduced and incubated for 30 min. After incubation, the remains of colloidal gold were washed with H_2O solution. Plasmon-77 and Plasmonotest-2K software were used to process the received data. Statistical processing of the results obtained was performed using STATISTICA 6 software.

Results

Firstly, the effectiveness of the substrate (transducer) of the SPR biosensor based on antibodies was checked (Fig.1). In particular, the optimal concentrations of spermine in test solutions have been determined in which a clear and stable response of the biosensor is observed. The optimal range of spermine concentrations was also determined. It was found that the optimal range of spermine concentrations when using a substrate with antibodies against spermine is in the range of 10-500 nM. In this case, the deviation of the resonant angle in this range is stable within 0.61-1.14 degrees. The overall sensitivity range ranges from 0.5-1000 nM, and the shift of the resonance angle varies from 0.04 to 1.98 degrees (Fig.1).

In addition, the effectiveness of two types of biosensor devices was tested – the SPR-6 optical biosensor manufactured at V. E. Lashkaryov Institute of Semiconductor Physics NAS of Ukraine V. M. Glushkov Institute of Cybernetics of National Academy of Sciences of Ukraine and biosensor device “Plasmontest” manufactured at V. M. Glushkov Institute of Cybernetics of National Academy of Sciences of Ukraine (Fig.2). In particular, it was determined that the difference in the deviation of the resonance angle when modifying the substrate of the biosensor with antibodies against spermine varies within 0.02-0.3 degrees (Table 1).

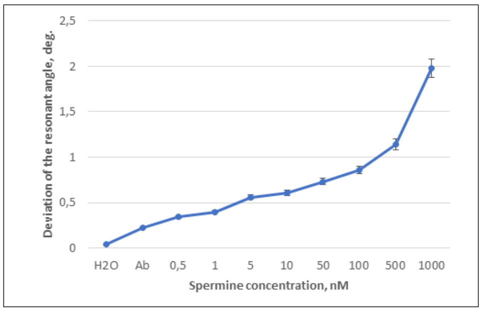

Figure 1: Determination of The Sensitivity Range of The Biosensor with The Substrate Modified with Antibodies Against Spermine

When determining spermine at concentrations of 0.5-500 nM, there is a slight increase in the deviation of the resonance angle in the SPR-6 biosensor compared to the Plasmontest biosensor, but the opposite situation is observed when determining spermine at a concentration of 1000 nM (Fig.2). However, these deviations are quite minor, so it can be observed that both types of biosensor devices are suitable and effective in determining spermine as a marker of PC.

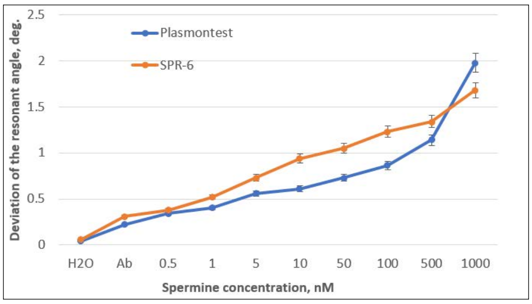

Figure 2: Comparison of The Efficiency of Spermine Detection Using the Biosensor Device “Plasmontest” And “Spr-6” On the Substrate Modified with Antibodies Against Spermine

In addition to testing the effectiveness of the substrate modified with antibodies against spermine, the effectiveness of the substrate modified with a solution of colloidal gold was tested. Gold nanoparticles with a diameter of 13-25 nm stabilized with a solution of sodium citrate have a high affinity for the amino groups of spermine, which causes aggregation of nanoparticles in the solution. According to the results obtained, it was found that the optimal range of spermine concentrations, both on the substrate of antibodies and colloidal gold, is within 10-500 nM. The general sensitivity range is within 0.5-1000 nM (Fig.3).

However, a clearer response of the biosensor, as well as a greater deviation of the resonant angle, is observed on the substrate treated with colloidal gold. Thus, the range of deviation of the resonance angle on the substrate with a layer of antibodies is in the range of 0.34-1.98 degrees, while on the substrate of colloidal gold, the deviation of the resonance angle is in the range from 0.38 to 3.33 degrees when determined by the Plasmontest biosensor device (Table 2).

Table 1: Comparison of The Magnitude of The Resonance Angle When Determining Spermine in Model Solutions on A Substrate of Antibodies of Two Biosensor Devices “Plasmontest” And “Spr-6”

	Plasmontest	SPR-6	Resonance angle deviation difference (degrees)
Analyte, nM	Resonant angle, degrees		
H_2O	62.34	62.36	0.02
Ab	62.52	62.61	0.09
0.5	62.64	62.68	0.04
1	62.7	62.82	0.12
5	62.86	63.03	0.17
10	62.91	63.24	0.33
50	63.03	63.35	0.32
100	63.16	63.53	0.37
500	63.44	63.64	0.2
1000	64.28	63.98	0.3

difference in the deviation of the resonance angle when using a substrate of antibodies and colloidal gold was 0.01-1.35 degrees in favor of the latter.

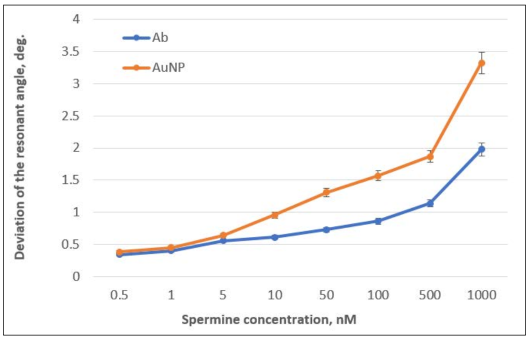

Figure 3: Deviation of The Resonance Angle When Introducing Spermine Within a Defined Concentration Range on A Substrate Treated With Antibodies Or Colloidal Gold

Compared with the previous device, the determination of the range of spermine concentrations using the SPR-6 biosensor device also showed better results when using a colloidal gold-modified transducer compared to anti-spermine antibody-modified transducer. So, the deviation of the resonance angle when using colloidal gold was in the range of 0.08-2.52 degrees, compared to the antibody-modified transducer, where the deviation was in the range of 0.06-1.68 degrees. The deviation difference of the resonant angle was in the range of 0.02 to 0.84 in favor of a transducer modified with a layer of colloidal gold.

Table 2: Comparison of The Deviation of The Resonance Angle When Determining Spermine in Model Solutions on A Substrate of Antibodies and Colloidal Gold of Two Biosensor Devices "Plasmontest" And "Spr-6"

Plasmontest	Transducer		Resonance angle deviation difference	SPR-6	Transducer		Resonance angle deviation difference
Analyte, nM	AuNP	Ab		Analyte, nM	AuNP	Ab	
H_2O	0.05	0.04	0.01	H_2O	0.08	0.06	0.02
Transducer	0.31	0.22	0.09	Transducer	0.29	0.31	0.02
0.5	0.38	0.34	0.04	0.5	0.44	0.38	0.06
1	0.45	0.4	0.05	1	0.73	0.52	0.21
5	0.64	0.56	0.08	5	1.31	0.73	0.58
10	0.96	0.61	0.35	10	1.46	0.94	0.52
50	1.31	0.73	0.58	50	1.60	1.05	0.55
100	1.57	0.86	0.71	100	1.74	1.23	0.51
500	1.87	1.14	0.73	500	1.96	1.34	0.62
1000	3.33	1.98	1.35	1000	2.52	1.68	0.84

Comparison of the efficiency of two types of biosensor devices when using a colloidal gold substrate showed that the deviation difference of the resonance angle varies from 0.03 degrees to 0.81 degrees. At the same time, for spermine concentrations in the range from 0.5 to 5 nM, the difference in the deviation of the resonance angle increased from 0.06 to 0.66 degrees in favor of the SPR-6 biosensor device.

However, starting from the spermine concentration of 10 nM to 500 nM, the difference in the deviation of the resonance angle begins to decrease significantly from 0.50 to 0.09 degrees, until it changes in favor of the Plasmontest biosensor device, where at a spermine concentration of 1000 nM, the deviation of the resonance angle reaches 0.81 degrees (Fig.4).

There is also a change in the color of model solutions during aggregation on the substrate of gold nanoparticles with spermine, which, probably due to a change in the optical properties of the substance and can affect the deviation of the resonant angle of the biosensor.

Thus, it can be concluded that a substrate treated with a layer of colloidal gold nanoparticles has a greater potential in determining spermine than a substrate treated with an antibody layer. In addition, both types of biosensor devices have shown high efficiency in the determined spermine, although with a slight advantage of the biosensor device "SPR-6".

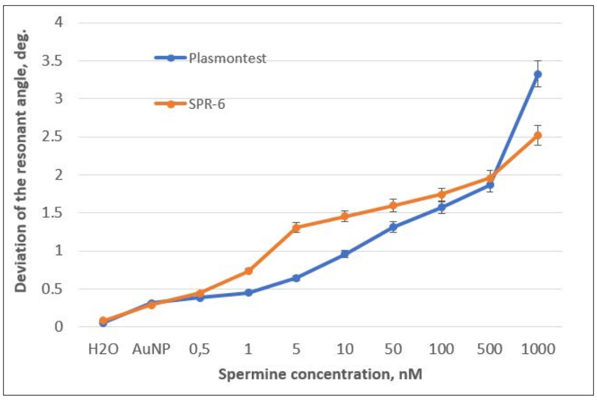

Figure 4: Comparison of The Efficiency of Spermine Determination Using the Biosensor Device "Plasmontest" And "Spr-6" On A Substrate Modified with A Solution of Colloidal Gold

Discussion

The ability to identify structural as well as functional changes in tissues during cancer has increased dramatically over the past two decades with the development of a variety of non-invasive methods, such as tomographic imaging [9]. Diagnosis of tumors using positron emission tomography (PET) is based on determining changes in metabolism in tumor tissues using appropriate markers [10]. Polyamines are aliphatic cationic amines that are involved in cell proliferation and cell differentiation. The relationship between the rate of spread of malignancy of tumors and a high level of polyamine biosynthesis has been established, which, in turn, is due to an increase in the exchange of polyamines and their predecessors [11]. In the prostate, the metabolism of polyamines occupies an important niche, since it synthesizes and accumulates a large number of polyamines, in particular spermine. Spermine was one of the first identified polyamines. High secretory activity of epithelial cells of the prostate lumen, maintains a relatively high level of spermine in the secretory fluid of a healthy prostate. Harrison et. al had shown that human prostate tissue synthesizes higher levels of spermine per day comparing with other tissues. Such a high rate of spermine synthesis is aimed at removing it into the secretion of the prostate gland, which makes the prostate the only human tissue where most of the synthesized polyamines are intended for export instead of maintaining cell proliferation [12].

At the early stage of PC, most foci of malignancy processes are local, and limited to individual organs and usually pass asymptomatic. There was a strong association between urinary/tissue spermine levels and the extent of tumor malignancy in patients with PCa. Higher spermine levels observed in normal prostate tissue and benign prostate hyperplasia (BHP), when in PC tissue a vast decrease of spermine due to changes of normal cell morphology and metabolism could be seen. Thus, a sharp decrease in the content of spermine in the prostate may indicate the transformation of prostate tissue from a benign phenotype to a malignant one [13]. In addition, Smith et al. reported that spermine inhibits tumor growth, both in vivo and in vitro [14]. Therefore, high levels of polyamines in the prostate, mainly spermine, serve as a potential candidate for biomarkers with high sensitivity to PCa. For the diagnosis of prostate cancer, the detection of spermine using optical biosensors looks promising and in particular due to the ease of use and high specificity due to the use of monoclonal antibodies against spermine and other polyamines. The variety of available nanomaterials opens the door to the development of new strategies for detecting biomarkers of PC [15]. Due to their internal optical, electronic and physicochemical properties, Au nanoparticles are widely investigated for use in biomedicine. Therefore, the use of optical biosensors to determine spermine looks promising approach in the diagnosis of cancer, prostate cancer.

Conclusions

1. For the biosensor analysis technique, two types of substrates were developed, which were modified with a layer of antibodies against spermine and a layer of sodium citrate stabilized colloidal gold.
2. Optimum concentrations of spermine when using a biosensor substrate based on colloidal gold and antibodies were determined, and corresponding to 10-500 nM.
3. The efficiency and comparison of the sensitivity of two types of biosensor devices "SPR-6" and "Plasmontest" in the determination of spermine in model solutions using substrates modified with an antibody layer and colloidal gold were determined.

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