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SHORT COMMUNICATION

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DETECTION OF POLYAMINES USING IMMUNOBIOSENSOR BASED ON ZINC OXIDE NANOPARTICLES

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***Aim*: To analyze the performance of biosensor based on nanoparticles of zinc oxide for the detection of spermine and spermidine in solution and in cell culture. *Materials and Methods*: Zinc oxide nanoparticles were used for preparing biosensor containing antibodies to spermine and spermidine. Polyamine concentration is solutions of spermine and spermidine as well as in lyophilisate of MCF-7 cells was measured by luminescence of the samples excited by laser beam at 380 nm. *Results*: The minimum concentration for the detection of polyamines in model solutions is 10 ng/ml, and maximum one is 100 ng/ml. A higher level of luminescence intensity of nanoparticles was found during analysis the polyamines in MCF-7 lyophilisate allowing for detecting polyamines at concentrations from 100 cells/ml to 100,000 cells/ml. *Conclusions*: The proposed biosensor system for determining the level of biogenic polyamines in cell lyophilisate using the optical properties of zinc oxide nanoparticles is promising for further improvement of the methodology and its implementation for detection and measurement of polyamines in biological systems.**

***Key Words*: MCF-7 cells, biosensors, photoluminescence, nanostructures, spermine, spermidine.**

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Biosensors are instrumental analytical tools for the rapid detection of various specific molecules or analytes. The biosensor contains a bioselective layer that reacts with a specific biological molecule, resulting in the corresponding physical signal (optical, chemical, electric, thermal, etc.) [1–6]. The type of the converter determines the method of measurement of the biosensor, its conditions, and limitations.

Recently, the attention of researchers is devoted to nanomaterials. Transducer effects of nanomaterials are due to small size, charge, permeability, surface structure and its high ratio to volume [7, 8].

The main advantages of the optical methods of detection are high precision and label-free concept. Optical biosensors working on the basis of absorption, photoluminescence, and surface plasmon resonance are quite promising devices for everyday use. In optical biosensors, the biological sensing element is connected to optical transducer system and the signal is based on absorption, luminescence or reflectance [9]. The success of the analysis depends on the formation of the bioselective layer by immobilizing the biological element of recognition (enzyme, receptor protein, probe molecule, cell-receptor, etc.) on the surface of the converter. This layer of biological recognition (functionalized surface) serves as the basis for binding an analyte (low molecular weight compound, protein, nucleic acid, or cell). Surface parameters of the transducer (effective surface area, roughness, porosity), physical and chemical properties (surface charge, energy, valence/conductance states, physical states, functional groups, hygroscopic nature) affect the formation of the bioselective layer [10]. The changes in the optical signal that arise when analyzing the binding allow for measuring the concentrations of the corresponding analytes.

One of the most promising materials among metal oxides is a semiconductor based on zinc oxide (ZnO) [11–16]. New technological methods of manufacturing ZnO nanostructure templates with a large surface area and additional properties for use in biosensor analysis have been developed. Nanostructured ZnO films are widely used as a template in electrochemical biosensors, since they showed good sensitivity and low detection limit of the analytes [17–19].

Deregulation of the level of polyamines and enzymes of their synthesis is often associated with pathological conditions, in particular, tumor growth, and their level in urine and plasma can be used as a diagnostic indicator of cancer. Previous studies have found that subtle ZnO films with higher internal stresses used in electrochemical biosensors show increased sensitivity to glucose due to the high efficiency of charge transfer. It is shown that ZnO nanostructures with a larger surface area provide a high level of immobilization of DNA on their surface [20].

The aim of the study was to analyze the performance of biosensor based on ZnO nanoparticles for the detection of spermine and spermidine in solution and in cell lyophilizate.

MATERIALS AND METHODS

***Preparation of biosensor surface*.** ZnO samples were provided by the Institute of Atomic Physics and Spectroscopy of the University of Latvia (Riga, Latvia). 20 µl of ZnO solution was deposited on glass plates 0.5×0.5 cm to form a ZnO-glass complex. The plates were dried at room temperature and annealed in a muffle oven at 400 °C for 2 h. ZnO-glass surface was further modified with polyallyl hydrochloride (PAH) (Sigma-Aldrich, USA) in order to increase sensitive surface. For this purpose, a solution of PAH was applied to a glass plate with ZnO and incubated for 20 minutes

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Abbreviations used: PAH – polyallyl hydrochloride.

at room temperature. The plate was then washed with distilled water and dried at room temperature. Following PAH treatment, the glass plates with ZnO layer were coated with solution of Protein A from *Staphylococcus aureus* (Sigma-Aldrich, USA), fixed with glutaraldehyde, washed after incubation period with 0.85% NaCl and coated with polyclonal rabbit antibodies against spermine (ab26975, Abcam, England) or spermidine (ab7318, Abcam, England). The next step was to apply BSA (Sigma-Aldrich, USA) on surface of glass plate followed by washing. After that, the plates were put in Petri dishes containing the moistened filter paper and stored at 4 °C till experiment.

Polyamine solutions and cell lyophilisate. Spermine and spermidine (Sigma-Aldrich, USA) solutions for determining the lower and upper threshold for sensitivity of the immunobiosensor were prepared at concentrations from 10 to 100 ng/ml in 0.85% NaCl. MCF-7 cells were cultured by conventional technique (Dulbecco's Modified Eagle Medium with 10% fetal calf serum, 1% l-glutamine, 1% penicillin and streptomycin at 5% CO_2), detached from the surface of flasks, and cell suspension (from 100 to 100,000 cells/ml) was freeze-dried in a protective medium (0.02 M PBS, 0.9% NaCl and 5% sucrose) at –60 °C for 18 h.

Assay for polyamine content. Measurements were carried out by transferring the modified glass plate (transducer) to the measuring cell of HR2000 spectrometer (OceanOptics, USA). The luminescence of the sample was emitted by laser beam at 380 nm. The readings were taken and converted in photoluminescence units. After the luminescence of ZnO-glass complex was measured, the samples to be assayed were placed a measuring cell in a volume of 20 µl, incubated for 10 min and the luminescence was measured at the point when the readings were stabilized. After completion of the measurements of each individual concentration, the measuring cell was rinsed with 0.85% NaCl solution and then the next analyte (polyamine or MCF-7 cell sample) was added with the increasing concentrations.

RESULTS AND DISCUSSION

The results of testing various concentrations of polyamines in model solutions and in MCF-7 cell lyophilizate are presented in the Table. For measurement of the concentration of polyamines (the sum of spermine and spermidine) in MCF-7 cells, the biosensor with antibody to spermine was used taking into account its cross-reactivity with spermidine). As control substance, a solution of 0.85% NaCl was used in concentrations corresponding to those used in the analysis of polyamines.

The results of the studies illustrate the dependence of the photoluminescence intensity of ZnO nanoparticles on the concentration of polyamines. There is a gradual decrease in the intensity of photoluminescence with an increase in the concentration of polyamines both in solutions and in cell lyophilizate. The decreased photoluminescence intensity with each new sample added to the measuring cell is due to the saturation of the free binding sites with polyamines and the blocking of all specific binding sites on the sensitive layer. Based on the data presented in Table, the distinct limits beyond which a biosensor can detect polyamines 10–100 ng/ml, however, it can be observed that the technique is capable of detecting polyamines in an amount less than 10 ng/ml.

This technique allows also detecting polyamines in low concentrations in about 100 cells/ml with the concentration of polyamines starting from 10 ng/ml. Comparing the luminescence intensity in polyamines with sample of 100,000 cells, the concentration of polyamines in that sample of cells was in the range of 70–100 ng/ml. Nevertheless, the values in photoluminescence units in samples of MCF-7 cell lyophilizate were higher than in samples of spermine and spermidine solutions. The luminescence intensity in MCF-7 cell lyophilizate was significantly higher even when compared with samples with minimum concentration of polyamines (10 ng/ml).

Thus, the use of ZnO nanoparticles allows for both qualitative and quantitative determination of the level of polyamines, both in the solution of polyamines and in MCF-7 cell lyophilizate. The methodological approach proposed does not require a large number of expensive reagents (gold, silver, as in biosensors based on surface plasmon resonance), which makes it more economical than known analogues. At the same time, it takes less time to prepare and carry out the analysis itself due to the lack of need for some operations. This provides an important opportunity to reduce the time to decide on the start of the development of malignant tumors, and the timely appointment of appropriate therapy. The developed method is new and has many unique features comparing with methods used for detection of polyamines (such as enzyme-linked immunosorbent assay, high performance liquid chromatography, or other optical biosensors). Time for the preparation of samples for takes up to 10–15 minutes, whereas for biosensors based on surface plasmon resonance it can take 30–40 minutes, and traditional enzyme-linked immu-

Table. Determination of the concentration of polyamines in model solutions and MCF-7 lyophilizate using a ZnO-based immunobiosensor

C, ng/ml	10	20	30	50	70	100
			Intensity of photoluminescence, PI, units			
Control	5000 ± 175.7	4500 ± 188.3	4200 ± 232.3	3900 ± 240.9	3700 ± 268.2	3400 ± 283.1
Spermidine	6500 ± 196.3*	6300 ± 188.3*	6200 ± 186.5*	6000 ± 182.6*	5850 ± 173.9*	5700 ± 168.6*
Spermine	6700 ± 222.1*	6450 ± 191.3*	6300 ± 188.3*	6100 ± 184.3*	5900 ± 175.5*	5800 ± 161.8*
C, cells/ml	100	500	1000	5000	10,000	100,000
MCF-7	7500 ± 196.3*	7000 ± 238.8*	6600 ± 257.5*	6300 ± 285.8*	6000 ± 344.5*	5900 ± 388.7*

Note: $*p < 0.05$ comparing with controls.

nosorbent assay methods of analysis, takes several hours. The proposed biosensor system for determining the level of biogenic polyamines using the optical properties of ZnO nanoparticles may be promising for its implementation with the aim of highly sensitive rapid and accurate detection and quantification of biogenic polyamines which may be of diagnostic value in oncology.

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