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Novel immune TiO_2 photoluminescence biosenors for leucosis detection

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Abstract:
Novel immune photoluminescent biosensor, based on TiO_2 nanoparticles, for retroviral leucosis detection has been developed. The photoluminescence spectra were excited by solid state laser with wavelength 355 nm and measured in the range of 370-800 nm. Original photoluminescence spectrum of TiO_2 nanoparticles showed wide maximum at 515 nm. The biosensitive layer was formed by immobilization of retroviral leucosis antigens on the surface of TiO_2 nanoparticles. Immobilization of antigens on TiO_2 surface led to UV-shift of photoluminescence spectrum and increase of PL intensity. The response to different concentrations of retroviral leucosis antibodies has been measured. The decrease of spectrum intensity and IR-shift were observed after antibodies adsorption on biosensor surface. The experimental dependences of maximum shift and intensity changes versus antibodies concentration were obtained.

Key words: TiO_2 nanostructures, immune biosensors, leucosis detection, photoluminescence

Introduction

Bovine leucosis – is the highly foetal neoplasia of the cattle characterised by the abnormality maturation process of the blood cells and the aggregation of the neaplastic lymphocytes in the lymph nodes [1]. Clinical signs are most commonly associated with the infection and they include weight loss, decreased milk production, lymphadenopathy and posterior paresis. Virus of the type C (retrovirus family from an oncovirus rod) is the causative agent of the disease. Virus contains the revertase and six antigen proteins, among of which is the superficial (an envelope protein) glycoprotein (gp51) and the inside protein (p21) [2].

Once acquired, viral infection is lifelong and is spread by the contact between the animals. At any age animals can be infected with bovine leukaemia virus (BLV), but tumour (lymphosarcomas) is seen typically in the animals over 3 years old. Infections are usually sub clinical; only 30-70% of the infected cattle develop the persistent lymphocytes and only 0.1-10% - develop the tumours [3].

The Epidemiological and the End Results Program of USA were used to perform a descriptive epidemiological study of the leukaemia in Iowa. The ecological relationships between the human leukaemia, the livestock populations and the bovine lymphosarcoma were investigated. There is a high positive correlation between the acute lymphoid leukaemia in the males and the cattle density. This relationship is higher for the dairy cattle than for the beef cattle. So, leukaemia is the potential problem from the both public health and economic perspectives [4].

Diagnosis of the BLV infection based on the clinical signs alone is difficult because of the wide range of symptoms. More then 20 various variants from the haematological, histological [1] and up to use of the polymerise chain reaction (PCR) [5, 6] as well as a different variants of the immunological methods, for example, the classical fluorescence [7], the immune diffusion [8], the radioimmune analysis [9], the immunoblot [5] were developed. The traditional immune methods have high specificity and sensitivity, but they take a lot of

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time, and require additional parameters such as the labelled molecules. This drawback can be overcome with the use of the modern instrumental analytical devices based on the biosensor technology.

TiO_2 is well known material for optical, catalytic and sensing applications. The development of various deposition techniques allows synthesis of novel titanium dioxide structures with dimensions on the nanometre scale. The decrease of the dimensions below certain levels may lead to the formation of quantum-size effects such as the absorbance edge shift and the room temperature photoluminescence (PL) peaks appearance [11]. Application of these effects, especially optical properties, for sensor technologies could be a novel trend of sensorics. Optical methods have good advantages such as higher preciseness and low energy consumption.

Early, we have developed the immune biosensor based on the SPR and intended for the bovine leucosis diagnostics [12, 13]. The traditional immune methods have high specificity and sensitivity, but they take a lot of time, and require additional parameters such as the labelled molecules [12]. This drawback can be overcome with the use of the modern instrumental analytical devices based on the biosensor technology [13].

Experimental

Previously, it was shown good capacities of SiO2 hydro gels, based on commercial SiO_2 nanofibers, for biosensors [11]. In the presented work commercial TiO_2 nanoparticles (Sigma Aldrich, particle size 32 nm) were used as biosensor template. TiO_2 nanoparticles were solved in water to prepare sols. TiO_2 layers were formed on glass substrates by dropping TiO2 sols and drying at room temperature. Post annealing treatment at 300 ^{0}C for 1 hour was provided to remove water from the samples. Structural properties of the obtained samples were studied with SEM method. Fabrication of sensitive layer was performed by immobilization of Ag on TiO_2 surface. TiO_2 nanostructures were exposed to water solution of leucosis antigens (Ag) for ten minutes and then were washed two times in distilled water and dried in air at temperature 40 ^{0}C. The backside of TiO_2 sample was sealed to prevent immobilization of Ag on it. Leucosis antibodies (Ab) were deposited on TiO_2-Ag surface from water solutions with different concentrations. Photoluminescence (PL) spectra were measured by setup, presented in Fig. 1. The luminescence was stimulated by UV laser LCS-DTL-374QT with excitation wavelength λ=355 nm. The emission spectra were amplified and recorded in the wavelength range 370-800 nm.

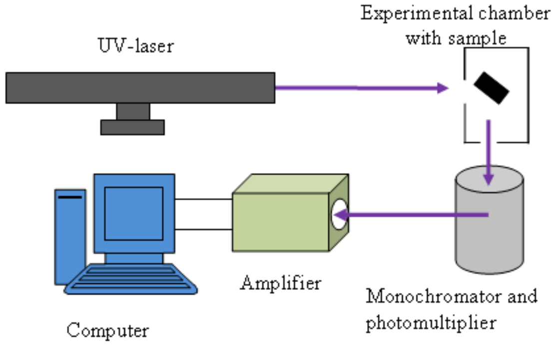

Fig.1. (a) Photoluminescence setup.

Results and discussion

SEM measurements showed that nanoparticles formed high surface area porous structure (Fig.1). The obtained structure was suitable platform for immobilization of biological species.

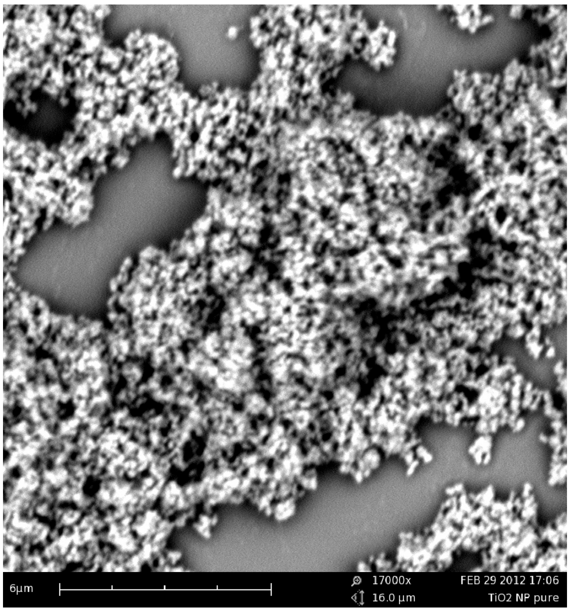

Fig.2. SEM image of TiO_2 nanostructures, deposited on glass.

PL spectrum of TiO_2 nanoparticles had broad peak centered at 514 nm (Fig.3). The immobilization of leucosis Ag on TiO_2 surface led to the changes in PL intensity and PL peaks positions after immobilization of Ag onto TiO_2 nanoparticles surface. It was found that after Ag immobilization PL spectra was shifted to shorter wavelengths. It can be a proof of formation links TiO_2-Ag. Increase of PL intensity could result from charge transfer between Ag molecules and conductance band of TiO_2. UV-shift of PL maximum can be explained by additional dipole-dipole interaction, what can change energetic position of recombination centres into TiO_2.

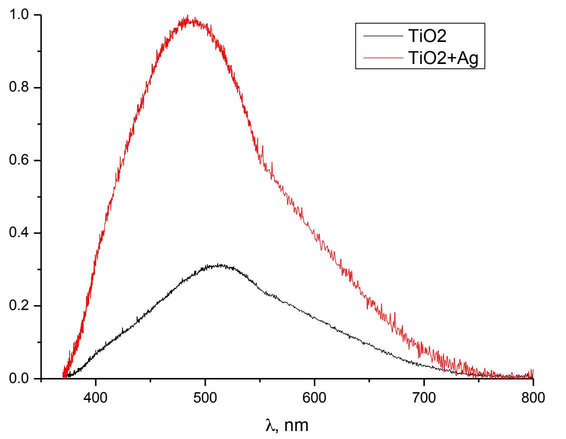

Fig. 3. PL spectra of TiO_2 nanoparticles before and after immobilization of antigens (Ag).

PL spectra of TiO_2-Ag biosensor, measured under different Ab concentrations are shown in fig. 4. It was found that PL intensity decreased with Ab concentration. At the same time, peak position moved to higher wavelengths.

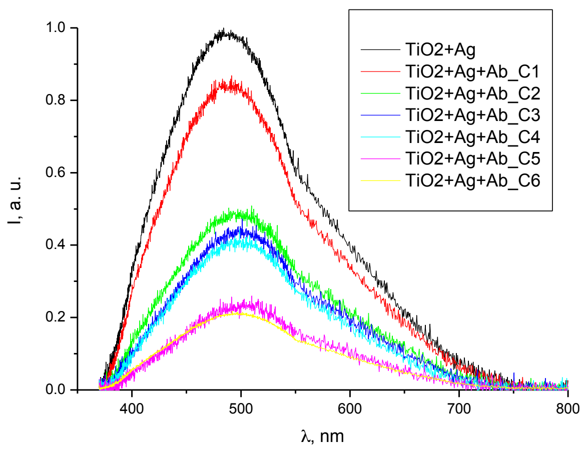

Fig. 4. PL spectra of biosensors under different concentrations of antibodies (Ab).

Thus, the biosensor response to leucosis Ab can be a function of two parameters: PL intensity and position of PL peak. To analyze the sensor response we calculated the changes biosensor signal *S* as

$$S = \frac{S_{Ag} - S_{Ab}}{S_{Ag}} \quad (1)$$

where S_{Ag} and S_{Ab} are PL peak's intensities of TiO2 nanostructures with immobilized leucosis antigens before and after interaction with leucosis antibodies, correspondently. The changes of peak position after adsorption of Ab were calculated in by following equation:

$$\Delta\lambda = \lambda_{Ag} - \lambda_{Ab} \quad (2)$$

where λ_{Ag} and λ_{Ab} are PL peak's positions of TiO_2 nanostructures with immobilized leucosis antigens before and after interaction with leucosis antibodies, correspondently. The results, obtained with the use of equations (1), (2) are plotted in figures 5 and 6, correspondently.

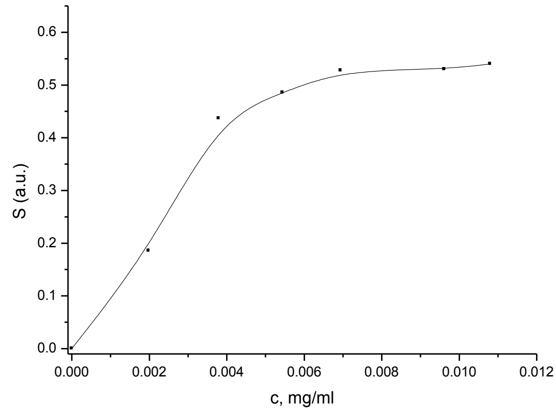

Fig. 5. Response of the biosensor signal to different concentrations of antibodies (Ab).

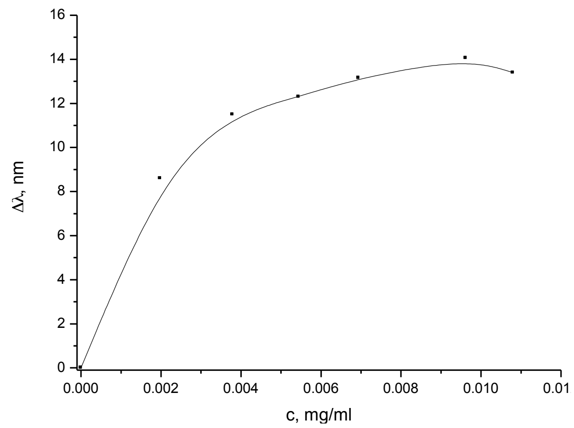

Fig. 6. The changes of PL peak position of the biosensor vs different concentrations of antibodies (Ab).

The analysis of obtained results showed that the changes of biosensor parameters had similar behavior. The obtained experimental curves increased at the range of Ab concentrations c 0.002-0.008 mg/ml. The further increase of Ab concentration led to saturation of the signal changes.

To determine the maximal sensitivity region of the studied biosensor, an experimental curve (S/c) vs c was plotted (Fig.7). The obtained curve had maximum at c=0.004 mg/ml. With the increase of Ab concentration the decrease of experimental curve was observed. Matching the region, where sensitivity decreased in 30% we can assume that optimal operating region for leucosis biosensors lays in the region of Ab concentrations 0.002-0.006 mg/ml.

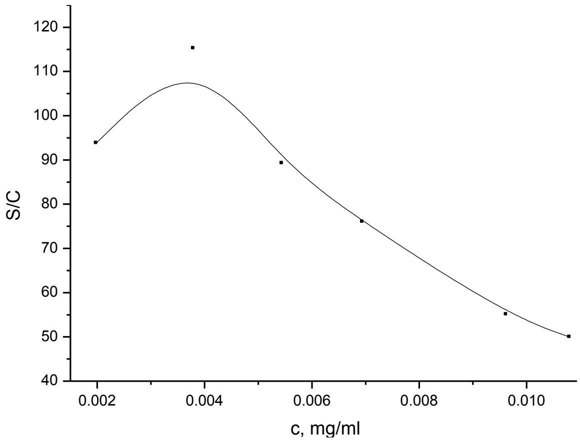

Fig. 7. Sensitivity of the biosensor vs different concentrations of antibodies (Ab).

Conclusion

Novel photoluminescence biosensor based on TiO_2 nanoparticles for leucosis detection has been developed. The biosensor response to leucosis antibodies is a function of PL intensity and peak position. The obtained biosensors can operate in the range of leucosis antibody concentrations 0.002-0.006 mg/ml.

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