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Immune Biosensor Based on Silica Nanotube Hydrogels for Rapid Biochemical Diagnostics of Bovine Retroviral Leukemia

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Abstract

The presented paper is devoted to investigation of properties of silica nanofibers hydrogels for biosensors applications. SiO_2 nanofibers were prepared by the electrospinning method from polymeric solutions, containing SiO_2 precursor, followed by a calcination procedure to obtain highly crystalline, pure inorganic nanofiber materials. The structural properties have been investigated by Scanning electron microscopy (SEM). Hydrogels were prepared by mixing of SiO_2 nanofibers in distilled water. Photoluminescence (PL) spectra (excitation with λ=266 nm) of SiO_2 hydrogels with immobilized antigens (Ag) and antibodies (Ab) in the range were studied in the region 370-620 nm. Experimental dependence of PL properties of SiO_2 hydrogels with immobilized Ag on Ab concentration was studied.

Keywords: hydrogels; silica nanotubes; biosensors.

1. Introduction

Bovine leucosis (BLV) – is disease caused virus of the type C (retrovirus family from an oncovirus rod). The ecological relationships between the human leukaemia, the livestock populations and the bovine lymphosarcoma had shown a high positive correlation between the acute lymphoid leukaemia in the males and the cattle density. So, leukaemia is the potential problem from the both public health and economic perspectives. More then 20 various variants from the haematological, histological and up to use of the

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polymerise chain reaction as well as a different variants of the immunological methods. The traditional immune methods have high specificity and sensitivity, but they take a lot of time, and require additional components such as the labelled molecules. This drawback can be overcome with the use of the modern instrumental analytical devices based on the biosensor technology [1].

Early we have developed [2] immune biosensor based on the surface plasmon resonance (SPR) and nanostructure silicon for the express revealing of some biochemical quantities and a special attention was paid the application of these approaches for the diagnostics of BLV. SPR based immune biosensor has some disadvantages connected with the high cost of the chips and the necessity to use not simple procedure of the preliminary transducer surface treatment from one site. From other site the SPR recorder has very high price. Now to overcome some of these disadvantages we try to apply others types of the optical biosensors for solving of the problem of the express biochemical diagnostics of BLV. Among others such biosensors that based on the silica nanotubes attract an especial attention [3].

The presented work describes rapid tests for bovine leucosis by measuring PL spectra of silica nanotubes hydrogels. Analysis of the changes of PL spectra against Bovine leucosis species was provided.

2. Experimental

Silica nanoubes powder, deposited by electrospinning method, was used in all tests. Structural properties of silica nanotubes powder have been investigated by Scanning electron microscopy (SEM) and X-ray diffractometry (XRD). XRD tests were measured by Rigaku Ultima XRD-setup (CuKα, λ=0.154 nm) in the range of 2θ angle 20-80°. Scanning electron microscopy FEI Nova has been used to obtain surface images of the investigated nanotubes.

Optical properties were studied with use of UV-VIS spectrophotometer Shimadzu UV-1700 in the range 350-1100 nm. Photoluminescence (PL) spectra were measured by setup, presented in Fig. 1. The luminescence was stimulated by UV laser LCS-DTL-382QT with excitation wavelength λ=266 nm. The emission spectra were amplified and recorded in the wavelength range 370-900 nm.

For hydrogels preparation 10 mg of SiO_2 nanotubes were solved in 50 ml of distilled water and stirred for 2 hours to form homogenic hydrogel. As sources of the antigens (Ag) it was used the mixture of the retroviral proteins. Blood serums from health and ill cows were kindly presented by this venture too. Ag was dissolved in 0.05 M tris-HCl buffer (pH 7.3) at the different concentration. Antibodies (Ab) were found in serum blood of ill cows. For testing 200 µl of silica hydrogel was mixed with 200 µl of Ag and in 20 minutes washed with distilled water 3 times. Then, antibody (Ab) was added to the SiO_2-Ag hydrogel. Concentration of Ab varied from 100% to 1%.

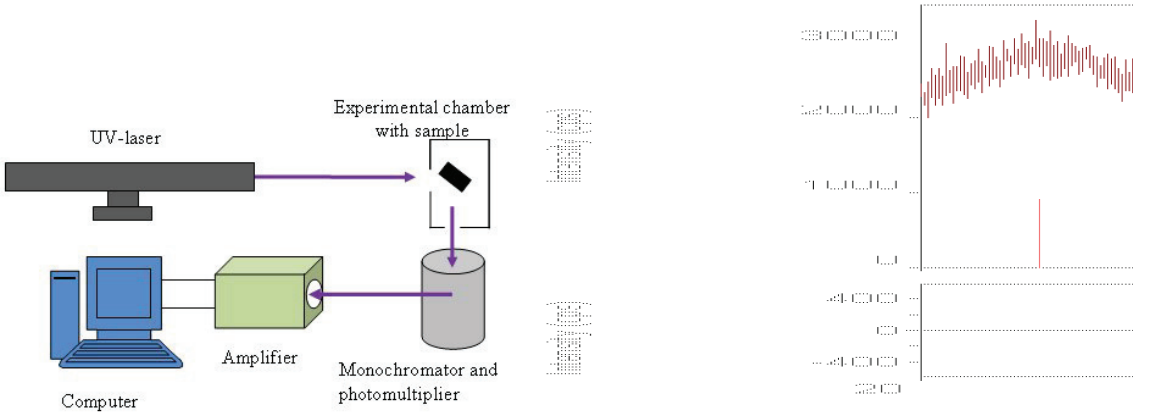
3. Results and discussion.

XRD spectrum of SiO_2 nanotubes is presented in Fig.1b. Single peak was found at 2θ=22 º, corresponding to SiO_2 [4]. The absence of other SiO2 peaks appoints that the obtained nanotubes have only one growth orientation similar to single crystals.

SEM image of the obtained nanotubes is shown in Fig.2a. From SEM measurements the linear dimensions of silica nanotubes have been calculated. The obtained samples had linear dimensions 250 nm in diameter and over 10 micron in length.

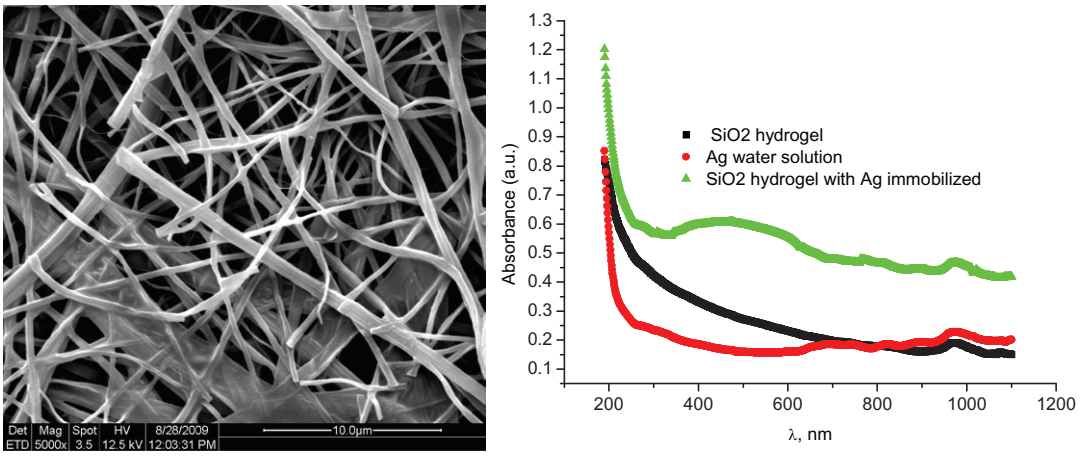
Optical transmittance of silica nanotubes hydrogels before and after immobilization of Ag was measured to detect interaction between surface of silica nanostructures and Ag species (Fig.2b). It is known that SiO2 is transparent in UV-Vis spectral region. Ag solution in water showed no peaks (Fig.2b),

whereas SiO2-Ag hydrogel showed wide peak at 400-600 nm, pointing to capture of Ag onto SiO_2 surface.



(a) (b)

Fig.1. (a) Photoluminescence setup; (b) XRD spectrum of SiO2 nanotubes.



(a) (b)

Fig 2. (a) PL spectra of SiO_2 hydrogels with immobilized Ag and Ab compounds; (b) PL spectra of SiO_2 hydrogels with immobilized Ag and Ab compounds under different Ab concentrations.

Photoluminescence spectra of SiO, SiO_2-Ag and SiO_2-Ag-Ab nanostructures are plotted in Fig.3a,b. It can be seen that initial PL peak of SiO_2 was shifted after immobilization of Ag and Ab onto silica nanotubes surface (Fig.3a).

Concentration dependence of PL spectra of SiO_2-Ag hydrogel on Ab is shown in Fig. 3b. It was found that PL intensity increased with Ab concentration. At the same time, peak position moved to higher wavelengths. Experimental curves of peak intensity at 438 nm and $\Delta\lambda$ ($\Delta\lambda=\lambda SiO_2$-Ab(1)-$\lambda SiO_2$) vs logarithm of concentration Ab are plotted in Fig.4. The obtained curved were non linear.

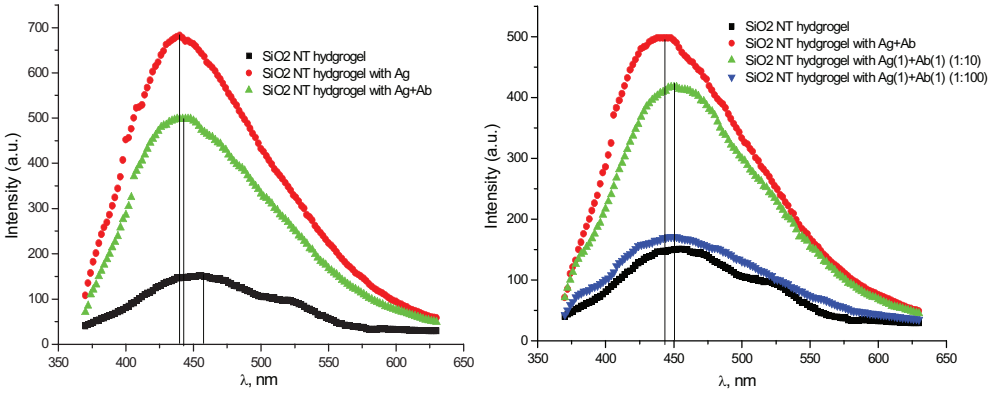


Fig 3. PL spectra of SiO_2 hydrogels (a) with immobilized Ag and Ab compounds; (b) PL spectra of SiO_2 hydrogels with immobilized Ag and Ab compounds under different Ab concentrations.

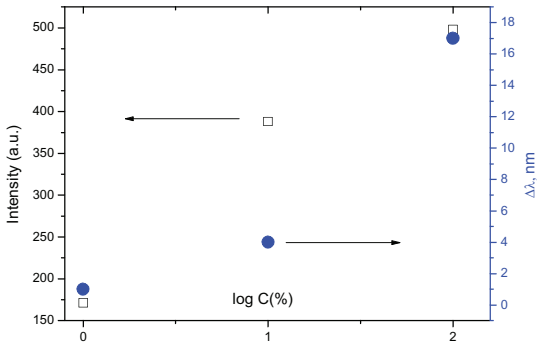


Fig 4. Dependence PL intensity of SiO_2 hydrogels (λ=438 nm) and wavelength shift Δλ on Ab concentration.

4. Conclusion

Thus, these results provide a basis for perspective application of the immune biosensor based on nanotubes for rapid biochemical diagnosis of such disease as leukemia viral cows.

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