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Development of Biosensor Chip for Detection of Specific Antibodies to HSV-1

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Abstract : In recent years, biosensor technologies based on the phenomenon of surface plasmon resonance (SPR) are becoming increasingly used in biology and medicine. Their application facilitates exploration in real time progress of binding of biomolecules and identification of agents that specifically interact with biologically active substances immobilized on the biosensor surface (biochips). Special attention is paid to the use of Biosensor analysis in determining the antibody-antigen interaction in the diagnostics of diseases caused by viruses and bacteria. According to WHO, the diseases that are caused by the herpes simplex virus (HSV), take second place (15.8%) after influenza as a cause of death from viral infections. Current diagnostics of HSV infection include PCR and ELISA assays. The latter allows determination the degree of immune response to viral infection and respective stages of its progress. In this regard, the searches for new and available diagnostic methods are very important. This work was aimed to develop Biosensor chip for detection of specific antibodies to HSV-1 in the human blood serum. The proteins of HSV1 (strain US) were used as antigens. The viral particles were accumulated in cell culture MDBK and purified by differential centrifugation in cesium chloride density gradient. Analysis of the HSV1 proteins was performed by polyacrylamide gel electrophoresis and ELISA. The protein concentration was measured using De Novix DS-11 spectrophotometer. The device for detection of antigen-antibody interactions was an optoelectronic two-channel spectrometer 'Plasmon-6', using the SPR phenomenon in the Krechman optical configuration. It was developed at the Lashkarev Institute of Semiconductor Physics of NASU. The used carrier was a glass plate covered with 45 nm gold film. Screening of human blood serums was performed using the test system 'HSV-1 IgG ELISA' (GenWay, USA). Development of Biosensor chip included optimization of conditions of viral antigen sorption and analysis steps. For immobilization of viral proteins 0.2% solution of Dextran 17, 200 (Sigma, USA) was used. Sorption of antigen took place at 4-8°C within 18-24 hours. After washing of chip, three times with citrate buffer (pH 5,0) 1% solution of BSA was applied to block the sites not occupied by viral antigen. It was found direct dependence between the amount of immobilized HSV1 antigen and SPR response. Using obtained biochips, panels of 25 positive and 10 negative for the content of antibodies to HSV-1 human sera were analyzed. The average value of SPR response was 185 a.s. for negative sera and from 312 to. 1264 a.s. for positive sera. It was shown that SPR data were agreed with ELISA results in 96% of samples proving the great potential of SPR in such researches. It was investigated the possibility of biochip regeneration and it was shown that application of 10 mM NaOH solution leads to rupture of intermolecular bonds. This allows reuse the chip several times. Thus, in this study biosensor chip for detection of specific antibodies to HSV1 was successfully developed expanding a range of diagnostic methods for this pathogen.

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