Silica Nanoparticles for Improving Efficiency of Virus-Like Particle Based Hepatitis B Vaccine

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***Abstract* – Adherence of hepatitis B virus-like particles (VLP) to silica (SiO_2) nanoparticles was explored for immunomodulation purposes. Optical absorbance measurements, transmission electron microscopy and fluorescence microscopy were employed to study the adherence. The results demonstrated that hepatitis B VLP + SiO_2 complexes were formed. Preliminary immunological experiments with vaccination of Balb/c mice with the VLP only and VLP + SiO_2 complexes were performed. The vaccination with VLP + SiO_2 complexes resulted in increase in antibody production in mice blood. The amount of antibodies produced strongly depended on the concentration of SiO_2 nanoparticles. The observed results suggest that SiO_2 nanoparticles can be considered as a promising material for improving efficiency of VLP-based vaccines against hepatitis B viral disease.**

***Index Terms* – hepatitis B, optical absorbance, silica nanoparticles, vaccines, virus-like particles.**

I. INTRODUCTION

Immunomodulation can be used for viral disease prevention where vaccine with immune response-modulating agents stimulates immune system to respond effectively to a viral disease. Virus-like particles (VLP) can be used as immune response-modulating agents. VLP have a protein shell derived from a real virus but do not have any genetic viral material inside it that means that VLP are not infectious. VLP containing vaccine is injected into blood vessels and VLP are delivered by bloodstream to specific cells where they stimulate antibody production. Treatment efficiency is higher when concentration of VLP near the specific cells increases. However, high concentrations of VLP in human body might result in side effects. To eliminate this, high concentration of VLP can be provided only in a vicinity of the specific cells.

In order to reduce the overall concentration of VLP simultaneously increasing the local concentration near the specific cells, a number of VLP can be attached to a nanoparticle that will act as a carrier of VLP to the specific cells. It is known that electrical charge is localized at the surface of VLP [1], therefore, the latter can be attached to the nanoparticle due to the electrostatic interaction if the nanoparticle has an opposite charge. Thus, the nanoparticle must have the ability for polarization. In addition, the nanoparticle must be harmless to human body. Both conditions are met by SiO_2 nanoparticles [2,3].

The aim of the study was to verify capability of SiO_2 nanoparticles to attach hepatitis B virus-like particles and to investigate the immune response of the organism after vaccination with VLP-based vaccine with added SiO_2 nanoparticles.

II. MATERIALS AND METHODS

Hepatitis B VLP were synthesized by the Latvian Biomedical Research and Study Centre. Certified SiO_2 nanoparticles were bought from the Sigma-Aldrich. Size of the nanoparticles was equal to 10 – 20 nm.

To study the capability of SiO_2 nanoparticles to attach VLP, the optical absorbance spectra of VLP, SiO_2 nanoparticles and VLP+SiO_2 mixture in buffer solutions were recorded and compared. The Thermo Spectronic Helios Gamma spectrophotometer was in use to record absorbance spectra at wavelengths 200 – 1090 nm. The role of the buffer solution was to keep pH constant. The buffer solution was prepared from 20 mM Tris-HCl pH 7.8, 5 mM EDTA, 150 mM NaCl, and 1 litre distilled water. NaCl concentration was equal to the concentration in the physiological solution. The concentration of SiO_2 nanoparticles was 1 mg in 1 ml of the buffer solution. The concentration of VLP was 12 µl in 1 ml of the buffer solution that corresponded to the optical absorbance value 1 (± 5%) at wavelength 260 nm. The value 12 µl was chosen after calibration procedure. It was known from experiments that Hepatitis B VLP have the optical absorbance maximum at 260 nm. Therefore, to make the calibration, optical absorbance for different concentrations of VLP was recorded and the concentration where the optical absorbance was equal to 1 at 260 nm was chosen for convenience of further result processing.

To verify the VLP+SiO_2 adherence, transmission electron microscopy (TEM) and fluorescence microscopy (FM) were employed. JEOL JEM-1200EX microscope was in use for TEM, and Leica DMI 3000 B microscope for FM.

To record fluorescence, VLP were marked with the green FITC agent, which forms covalent bonds with VLP amino acids. Fluorescence was excited at 490 nm and detected at 515 nm.

Preliminary immunological experiments were performed to study humoral response of Balb/c mice after vaccination with the VLP+SiO_2 complexes. The vaccination was made on days 0, 14 and 28. Mice from the control group were vaccinated with VLP only diluted into sterile phosphate-buffered saline. Two weeks after the 3rd immunization (on the day 42) all animals were bled and anti-HBc antibody response was detected using the direct ELISA test.

III. RESULTS AND DISCUSSION

The optical absorbance spectra of the solutions (SiO_2, VLP, VLP+SiO_2) were tested on time stability. To test the time stability, the absorbance was recorded at once after preparation of the solutions and after 24 hours (Fig.1).

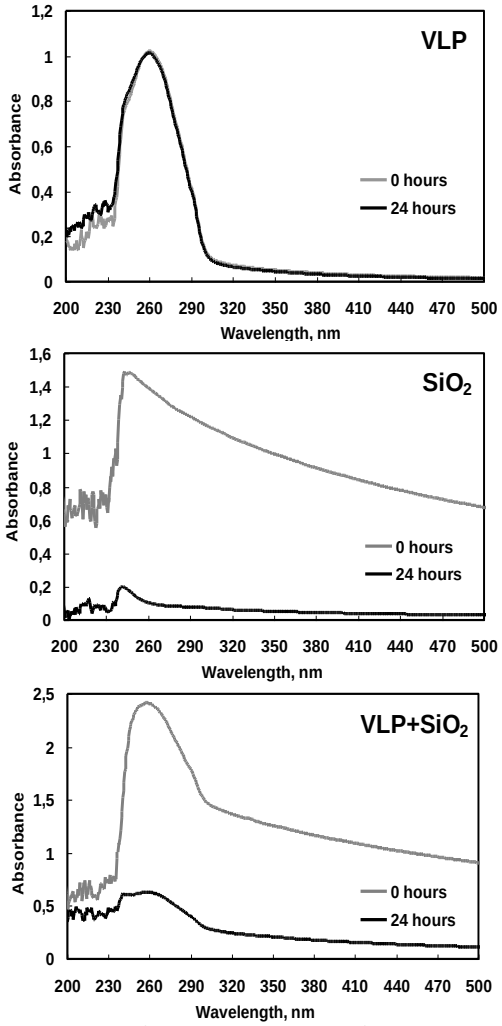

Fig. 1. Time stability of the optical absorbance of the VLP, SiO_2 and VLP+SiO_2 solutions.

The results demonstrated that the optical absorbance of the SiO_2 and VLP+SiO_2 solutions decreased after 24 hours and precipitations formed at the bottom of the test-tube. The precipitations could form due to gravitation forces which deflect the nanoparticles towards the bottom of the test-tube. However, the absorbance of the VLP solution did not change after 24 hours and no precipitations formed. Therefore, it was possible to suppose that VLP remain in suspended state in the buffer solutions at least within 24 hours.

The optical absorbance of the VLP+SiO_2 solution recorded in the experiment was compared with the theoretical optical absorbance value in order to see if VLP interact with SiO_2 nanoparticles. According to the spectrophotometry laws, the experimental and theoretical values must be equal if no interaction between the particles exists. To calculate the theoretical value, the absorbance of the VLP solution recorded experimentally at 260 nm was summed up with the absorbance of the SiO_2 solution recorded experimentally at 260 nm. Results demonstrated difference between the theoretical and the experimental values (Fig.2). The difference becomes more pronounced when the time given for VLP and SiO_2 interaction increases. That proves that VLP adhere to SiO_2 nanoparticles.

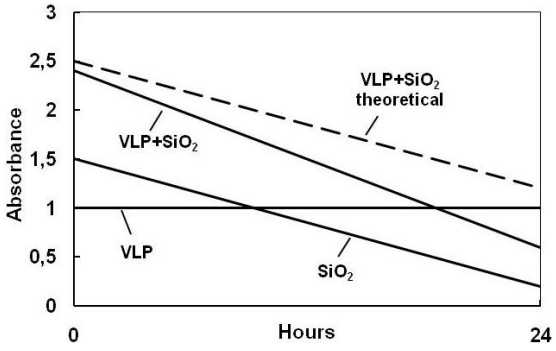

Fig. 2. Comparison between the experimental (solid line) and theoretical (dashed line) optical absorbance (at 260 nm) of the VLP+SiO_2 solution.

Both TEM (Fig.3) and FM (Fig.4) show the adherence of VLP to SiO_2 nanoparticles.

In case of FM, the VLP solution without the nanoparticles has homogeneous fluorescence. When SiO_2 nanoparticles are added, VLP adhere to them and fluorescence exists only in areas where VLP adhere to SiO_2 nanoparticles.

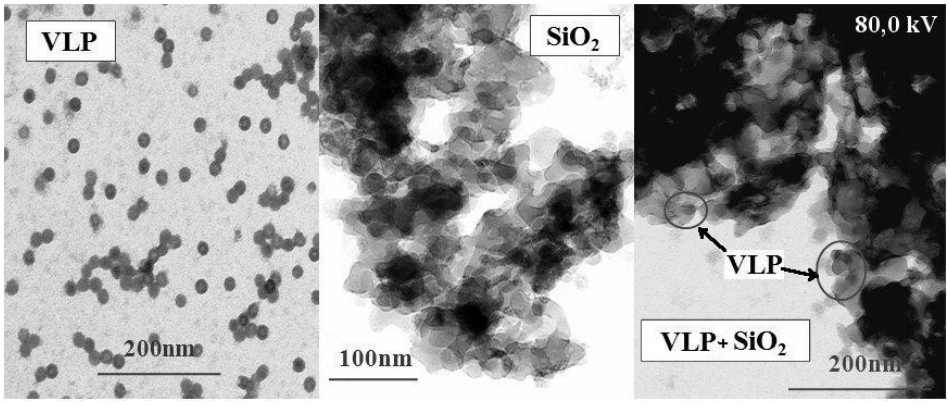

Fig. 3. TEM micrographs of the VLP, SiO_2, VLP+SiO_2 solutions.

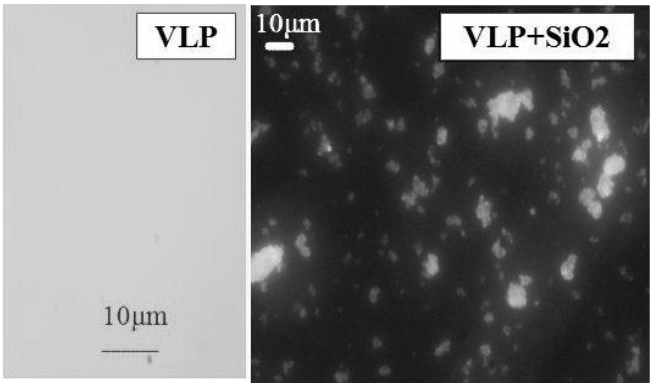

Fig. 4. FM micrographs of the VLP and VLP+SiO_2 solutions.

The results of the preliminary immunological experiment demonstrated that amount of antibodies produced in Balb/c mice blood depended on concentration of SiO_2 nanoparticles in the VLP+SiO_2 solution (Fig.5). The dose of VLP in the solutions was kept constant and was equal to 25 μg but concentration of SiO_2 nanoparticles varied thus resulting in different amounts of the VLP+SiO_2 complexes. VLP without SiO_2 nanoparticles induced lower antibody response than in case of the VLP+SiO_2 solution.

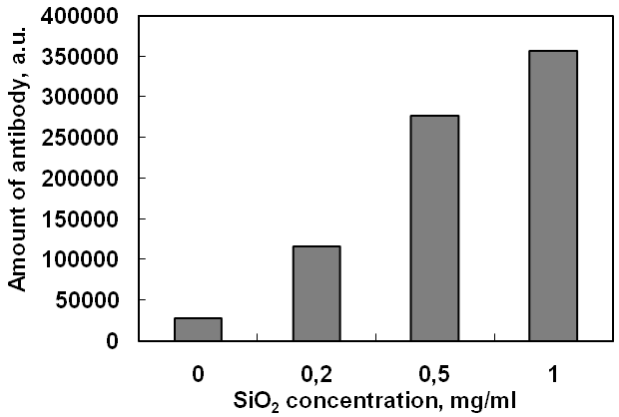

Fig. 5. Influence of concentration of SiO_2 nanoparticles in the VLP+SiO_2 solution on antibody production in blood of Balb/c mice.

Fig. 6 shows increment in amount of antibody production in mice blood in dependence on concentration of SiO_2 nanoparticles in the VLP+SiO_2 solution.

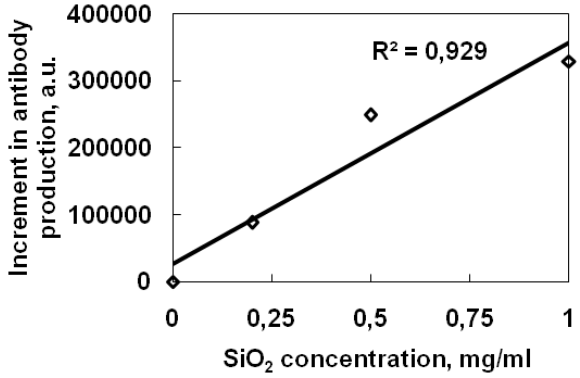

Fig. 6. Increment in antibody production in dependence on concentration of SiO_2 nanoparticles in the VLP+SiO_2 solution.

To calculate the increment value, the amount of antibody produced after vaccination with VLP only was subtracted from amount of antibody produced after the vaccination with VLP+SiO_2 solution. The results demonstrated that there was a good linear correlation (R-squared value was equal to 0,929) between concentration of SiO_2 nanoparticles and amount of antibodies produced in mice blood.

Optical absorbance and microscopy measurements prove that there is physical adherence between hepatitis B VLP and SiO_2 nanoparticles. The results of the immunological experiment evidence that vaccination with the VLP+SiO_2 complexes results in positive response in blood of Balb/c mice. It allows considering SiO_2 nanoparticles to be an effective material for efficiency improvement of VLP-based hepatitis B vaccines.

IV. CONCLUSIONS

1. Optical absorbance measurements, transmission electron microscopy and fluorescence microscopy demonstrate that there is a physical adherence between hepatitis B VLP and SiO_2 nanoparticles.
2. The correlation exists between increase in concentration of SiO_2 nanoparticles in the hepatitis B VLP + SiO_2 mixture and amount of antibodies produced in blood of Balb/c mice.
3. SiO_2 nanoparticles can be considered an effective material for efficiency improvement of VLP-based hepatitis B vaccines but further immunological studies are required.

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