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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: EM RESEARCH RELATED TO MEDICINE

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Abstract P-40: The Shape and Size of the Recombinant Virus-like Particles Were Checked by Means of Electron Microscope

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Background: Nucleocapsid protein of hepatitis-B virus (HBcAg) recombinantly synthesized in prokaryotic and eukaryotic cells is known to be capable to self-assemble into highly immunogenic stable viral-like particles (VLP) of icosahedral shape with a characteristic size of 32 nm (Schödel et al., 1994; Murray and Shiau, 1999). The VLP formation is tolerant to the insertion of some artificial epitopes to N- and C-termini of HBcAg monomer and also into major insertion region (MIR), forming a spike on the surface of VLP (Tordjeman et al., 1993, Peyret et al., 2015).

Methods: We have investigated the possibility of heterologous expression of *de novo* designed gene coding the first 148 amino acid residues of HBcAg (Pumpens and Grens, 1999). The gene was specially designed to be suitable for the insertions of genes coding fluorescent proteins, which are desired for the studies of VLP distribution in tissues by confocal microscopy. Gene was optimized for overexpression in *E. coli* producer strains and special attention was taken to obtain a simple purification scheme, which reliably reduces the amount of pyrogens in purified VLP.

The MIPT scientific platform of electron microscopy equipped with the transmission electron microscope Tecnai Polara G2 (Thermo Scientific (FEI)) was used. Carbon-coated (Lacey Carbon and 10 nm thin carbon) copper 200 mesh grids were treated with glow-discharge and coated with VLP suspension in deionized water. The samples were stained with uranyl acetate solution, air-dried, and inspected at the accelerating voltage of 300 kV.

Results: The 32 nm size of heterologously synthesized VLP was successfully proved, and spherical shape was seen using negative contrasting.

Key Words: hepatitis-B virus • electron microscopy • VLP

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