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PRODUCTION OF INFLUENZA VIRUS-LIKE PARTICLES BY INSECT CELLS AND REMOVAL OF BACULOVIRUS FROM VIRUS-LIKE PARTICLES AND OTHER EXTRACELLULAR VESICLES

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Enveloped virus-like particles (eVLPs) are a complementary strategy for the manufacturing of vaccines against enveloped viruses. The baculovirus expression vector system (BEVS) in insect cells is an attractive and widely used platform for the expression of eVLPs. Inherent to this system is the co-expression of baculoviruses and other extracellular vesicles, which we could nicely demonstrate by high resolution electron microscopy on one of our expression supernatants (Figure 1). Such heterogenous sample mixtures pose a major bottleneck for effective downstream processing of insect cell-expressed enveloped virus-like particles. eVLPs, baculoviruses and extracellular vesicles are similar in size and share a lot of common surface properties which make chromatographic separation difficult. In addition, immune responses against baculoviruses have been reported in humans, therefore efficient removal is mandatory.

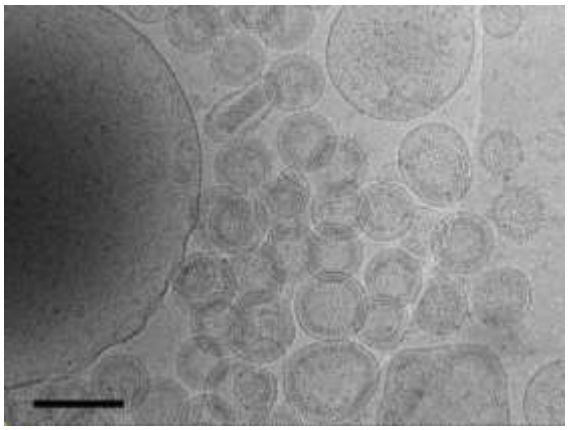

Figure 1– High resolution electron microcopy demonstrating the diversity of particles produced by the cell baculovirus expression vector system

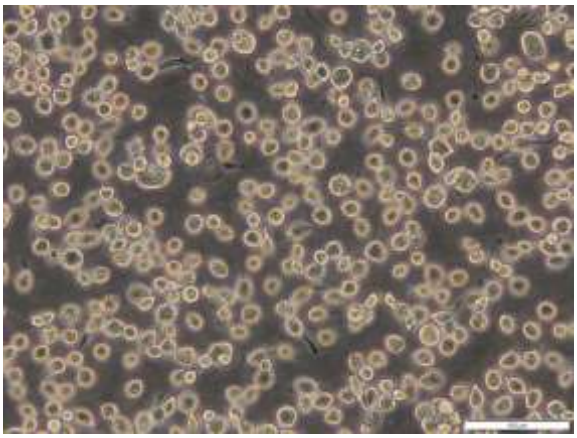

Figure 2 – The Tmns42 insect cell line, a derivative of High Five cell line (Hi5) Trichoplusia ni (Lepidoptera: Noctuidae) cabbage looper (ovaries) adapted to serum-free medium in suspension culture

We produced influenza HA-Gag virus-like particles by baculovirus infection of our Nodavirus-free *Trichoplusi ni*-derived *Tnms*42 insect cell line (Figure 2) in 10-liter bioreactors using suspension cell technology. A fast and simple purification method for these VLPs with simple processing of the feed and using one chromatography step has been developed. The insect cell culture supernatant was endonuclease-treated and filtered before it was directly loaded onto a polymer grafted anion exchanger, the DEAE Fractogel. Due to the much higher charge density on VLPs in contrast to soluble proteins, it is feasible to directly load and bind VLPs on ion-exchangers, while the majority of the host cell proteins and DNA is found in the flow through. After washing the unbound material, separation of baculovirus and VLPs was achieved by linear gradient elution, with VLPs eluting prior to baculovirus. A 4.2-log clearance of baculovirus from VLPs was achieved as measured by TCID50 infectivity assay. Effective clearance was further shown by the absence of the baculovirus-specific nucleocapsid protein vp39 and budded baculovirus virions determined by electron microscopy in the VLP fractions. Approaches for the implementation of successful downstream processes for enveloped virus-like particle-based vaccines and scale-up strategies for rapid process scale-up (ie. in a pandemic setting) will be discussed.