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Advances in bioprocessing, analytics and formulation of influenza HA-VLP vaccine candidates produced by insect cells

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ADVANCES IN BIOPROCESSING, ANALYTICS AND FORMULATION OF INFLUENZA HA-VLP VACCINE CANDIDATES PRODUCED BY INSECT CELLS

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The emergence of new influenza strains demands the continued development of novel, flexible, and scalable platforms for vaccine production. In this study, we describe advancements in the manufacturing process of influenza hemagglutinin (HA)-displaying virus-like particle (VLP)-based vaccines produced by insect cells, from upstream and downstream processing to analytics and formulation.
Aiming to improve influenza HA-VLPs production, evolutionary engineering and process intensification have been applied. Adaptation of stable Sf-9 cells producing HA-VLPs to hypothermic growth resulted in up to 12-fold higher expression. Likewise, adaptation of parental High Five cells to neutral pH induced a 3-fold higher specific HA-VLPs production rate following infection with baculovirus. In both case studies, the adaptation process had no impact on VLPs activity and morphology. Noteworthy, stable adapted Sf-9 cells could be cultured in perfusion (up to $100x10^6$ cell/mL) and continuous (~$20x10^6$ cell/mL) operation modes with cell-specific productivity similar to batch mode.

A broadly applicable filtration-based approach was developed to purify HA-VLPs independently of HA group and subtype, and of particle valency (i.e. monovalent or polyvalent VLPs). This strategy uses a cascade of ultrafiltration and diafiltration steps, allowing product recovery of 80%, with improvements in process costs, scalability and time when compared to a standard chromatography-based approach.
The robustness and generic applicability of an array of biophysical and biochemical methods (assessing particle size distribution, purity, surface charge, morphology and thermal stability) for HA-VLPs characterization has been successfully demonstrated. These methods allowed to cope with the different complexity of mono- and penta-valent VLPs, comprising HA from diverse groups (A and B) and subtypes (H1 and H3).
Finally, storage of influenza HA-VLPs was enhanced using a water-free formulation composed of a trehalose-glycerol (TGly) natural-deep eutectic solvent system. TGly allowed to reduce the rate of HA degradation and maintained VLPs' physical integrity upon storage at 50 °C. In addition, HA-VLPs were stable in TGly for slightly over one month at room temperature (20-23 °C), demonstrating the potential of this formulation for storage at non-refrigerated conditions.
Overall, the advancements herein described can be used to assist and/or accelerate influenza HA-VLPs vaccines development.

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