TOWARDS CONTINUOUS BIOPROCESSING OF LENTIVIRAL VECTORS

Sven Ansorge, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Sven.ansorge@nrc.ca
Maurizio Cattaneo, Artemis Biosystems Canada, Inc., Montreal, Canada
Anja Rodenbrock, National Research Council Canada, Montreal, Canada
July Dorion-Thibaudeau, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Stéphane Lanthier, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Sonia Tremblay, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Aziza Manceur, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Louis-Patrick Gagnon, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Krishna Raj Tiwari, Human Health Therapeutics, National Research Council Canada, Montreal, Canada
Rénald Gilbert, Human Health Therapeutics, National Research Council Canada, Montreal, Canada

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Lentiviral vectors (LV) represent a key tool for cell and gene therapy applications. The production of these vectors in sufficient quantities for clinical applications remains a hurdle, prompting the field toward developing suspension processes that are conducive to large-scale production. Advanced upstream bioprocessing approaches will need to be complemented by appropriate downstream processes in order to reduce overall manufacturing costs and address the current viral vector supply gap.

In this study, stable HEK293 producer cell lines were employed that grow in suspension, thus offering direct scalability, and producing a green fluorescent protein (GFP)-expressing lentiviral vector in the 10^{6-7} transduction units (TU)/mL range in batch culture without optimization. HEK293 stable producer cells were retained in 3 L bioreactors operated in perfusion mode using either a BioSep acoustic cell filter (Applisens), an XCell™ ATF system (Repligen) or a VHU™ Perfusion Filter (Artemis Biosystems). Cultures were grown up to 1 – 1.5 $\times 10^6$ cells/mL in batch mode. Perfusion was started at 0.5 volume of medium per reactor volume per day (VVD) and induction was carried out after reaching the targeted cell density of 5 $\times 10^6$ cells/mL. Perfusion was then continued at 1 VVD with fresh medium containing inducers for 3 – 4 days. In all perfusion runs, harvests were collected and the LV-containing supernatant was kept on ice or at 4ºC until clarification (once daily) and subsequently stored at -80ºC until quantification using the GTA assay. We are currently working on bioprocess development integrating this upstream process with suitable downstream approaches supported through the use of process development-enabling analytical methods.

Our study demonstrates that LV production in perfusion mode using the VHU filter outperformed our routine perfusion approach using an acoustic cell filter. Cells were retained in the bioreactor while LV particles passed through the filtration device with the harvest. Using this novel device, the cumulative functional LV titers were increased by up to 30-fold compared to batch mode, reaching a cumulative total yield of >2 $\times 10^{11}$ TU/L of bioreactor culture. This approach is easily amenable to large scale production and commercial manufacturing. Purification processes used to manufacture LVs need to be tailored to the unstable nature of LVs to counter vector instability and yields need to be improved through process optimization, such as the application of novel purification methodologies in continuous or semi-continuous mode. We will describe what DSP strategy we will use to most effectively integrate up- and downstream processing for lentiviral vectors. We also expect that our bioprocessing strategy will be transferable to other modalities having similar properties than LV.