Minimizing Lentiviral Vector Loss

Optimizing Purification Using an Innovative Nanofiber Adsorbent

with Sujeong Yang

Lentiviral vectors (LVVs) are crucial for long-term and stable gene expression to both dividing and nondividing cells. However, LVVs are difficult to process because of their high sensitivity to certain factors such as low pH, high salt, temperature changes, and shear forces. Sujeong Yang (senior research and development scientist at Astrea Bioseparations) introduced the LentiHERO platform, which helps users address challenging LVV purification steps, during a recent BPI webinar. The platform incorporates composite electrospun AstreAdept nanofibers, which use an expansive flow path and mild conditions to circumvent purification difficulties and achieve high yields of functional LVVs.

Yang's Presentation

Scalable strategies are necessary to remove the high levels of contaminants, such as host cell proteins (HCPs) and double-stranded DNA (dsDNA), that are found in suspension cultures. Doing so requires the development of clarification steps prior to purification. Yang's team conducted laboratory-scale studies to improve clarification of suspension LVV feedstocks. They examined the effects of centrifugation, microfiltration, and additional buffer exchanges (BXs) via ultrafiltration and diafiltration (UF/DF) on LVV yield and quality. The Nereus LentiHERO spin column was used as a screening tool for LVV capture. The optimized clarification step was then compared with other primary clarification methods, such as depth filtration with or without diatomaceous earth (DE). The final optimized workflow was confirmed using a fast protein liquid chromatography (FPLC)–compatible LentiHERO capsule to represent a manufacturing process.

Clarification Studies: The first study determined optimal BX volumes relative to LVV feed volume for UF/DF. Lentiviral feed was clarified using 1–3× volumes of exchange buffer to the feed volume, with 1× BX volume demonstrating minimal loss of the infectious titer (TU/mL), with >40% removal of HCPs and dsDNA. Larger BX volumes greatly decreased impurities, albeit along with a significant loss of infectious LVVs. These feeds were submitted for purification with the Nereus LentiHERO system to optimize infectious LVV recovery. 1× BX volume to the feed resulted in the highest infectious titer with 75% TU recovery, so that protocol was adopted for the UF/DF step.

A second study focused on reducing shear forces during primary clarification from the workflow start. The effects of centrifugation speeds, molecular weight cut-offs (MWCOs), and UF filters on fresh harvests were examined. The feed, clarified by centrifugation at 1,500*g* followed by BX with 100-kDa UF, showed minimal loss of infectious particles. Minimizing processing time is essential to maintain infectivity potential. Final recovery was assessed using the Nereus LentiHERO system for LVV capture. It enabled purification of six conditions in <25 minutes. The combination of 1,500*g* centrifugal speed, 0.45-µM microfiltration, and BX with 100-kDa UF gave optimal conditions for final recovery under low-shear conditions.

The third study compared the optimized clarification step with primary clarifications of centrifugation at 1,500*g* followed by microfiltration and depth filtration with or without DE. These results confirmed that the optimized clarification step minimized the loss of infectious LVVs postclarification with 86% TU recovery. That resulted in the highest recovery of infectious LVVs and highest removal of HCPs and dsDNA after purification.

Optimized Workflow: The crude LVV feedstock was purified with FPLC using a weak anion-exchange LentiHERO capsule. The final viral-genome recovery from the purified LVVs was 70%, and functional LVV recovery was 53% as measured by a Jurkat cell transduction assay. The process removed 98% of HCPs and 82–85% of dsDNA. Purification with the LentiHERO platform gave a high recovery yield and purity from LVV feedstocks generated from both adherent and suspension cells. Yang predicted that a 1-L unit of nanofiber could process a 200-L bioreactor in a single pass, helping to satisfy the current demand for larger-scale LVV production.

Questions and Answers

What are the unique properties of the AstreAdept nanofiber? Our nanofiber is composed of two different materials that provide strength and flexibility, which facilitate routine device scale-up. Additionally, the flow path is designed to enable a low-shear environment favorable for LVV, even at high flow rates.

Why does the LentiHERO FPLC device increase purification yield? This radial device enables fast processing times under mild conditions, with high binding capacities, which together increase purification yield.

How long would it take to operate a LentiHERO spin column as a screening tool? Each run takes <25 minutes, and multiple columns can run at the same time depending on the capacity of a centrifuge rotor.

Find the full webinar online at www.bioprocessintl.com/category/webinars.