TOWARDS AN INTEGRATED CONTINUOUS MANUFACTURING PROCESS OF ADENO-ASSOCIATED VIRUS (AAVs)

João P. Mendes, IBET, Portugal
jmendes@ibet.pt
Ricardo J.S. Silva, IBET, Portugal
Cristina Peixoto, IBET, Portugal
António Roldão, IBET, Portugal
Manuel J.T. Carrondo, IBET, Portugal

Key Words: Integrated processing, Perfusion bioreaction, Multi-stage TFF, Multi-column continuous chromatography.

Adeno-associated viruses (AAV) are one of the most applied vectors for in vivo gene delivery. However, depending on the medical condition to treat, the annual vector demand can reach up to 5×10^{20} VG placing substantial pressure on manufacturing processes. The work herein reported focuses on the steps followed toward a continuous manufacturing scenario as a pathway to overcoming the bottlenecks in the production and purification stages.

Bioprocess intensification was explored using Alternating Tangential Flow (ATF®) and Tangential Flow Depth Filtration (TFDF®) devices to enable AAV expression at high cell density (HCD). The obtained virus yields matched or surpassed batch cultures (up to $8x10^4$ VG/cell). Continuous harvest and clarification of the bioreactors were also explored using these cell retention devices. TFDF® enabled high throughputs and viral genome recoveries of >800 LMH and >90%, respectively.

For large-scale manufacturing, the reduction of handled volumes and processing time are critical, and in this sense, we implemented a customizable multi-stage tangential flow-filtration (MSTFF) strategy to concentrate virus particles before intermediate purification in a single passage. This was enabled by the screening of different cassette configurations, flow rates, path lengths, and conversion percentages. The optimized MSTFF setup enabled a titer increase of 5-fold with AAV recoveries above 92%. Finally, AAV capture was further purified with an affinity chromatographic method using Periodic Counter-Current Chromatography (PCC) with 4 columns. The design of the PCC process enabled the decrease of resin and buffer consumptions while demonstrating the potential to improve productivity and processing time. This approach yielded over 85% recovery in viral particles and considerable impurity reductions (> 98% DNA, and > 99% total protein) through consistent processing runs of multiple cycles.

In summary, the potential of process intensification for AAVs to overcome upstream and downstream bottlenecks was demonstrated through the use of perfusion strategies, new clarification methods, multi-stage TFF, and continuous chromatography. This enabled not only improved virus recovery yields, and productivity, but also the reduction of footprint and processing time, creating new opportunities for evolving the current processes beyond the batch paradigms and limitations.