Membrane Technology Significantly Intensifies Gene Therapy Manufacturing

Recombinant adeno-associated viral (AAV) vectors have rapidly emerged as the leading platform for *in vivo* delivery of gene therapies. AAV vectors have low immunogenicity and can be delivered to many kinds of tissues, enabling the treatment of a wide variety of genetic disorders. The first U.S. Food and Drug Administration (FDA)-approved AAV therapy came in 2017 with LUXTURNA, developed by Spark Therapeutics. Since then, successive product approvals have propelled the global market for AAV-based therapies beyond $1 billion. However, the high cost of AAV therapies severely limits access to these drugs.

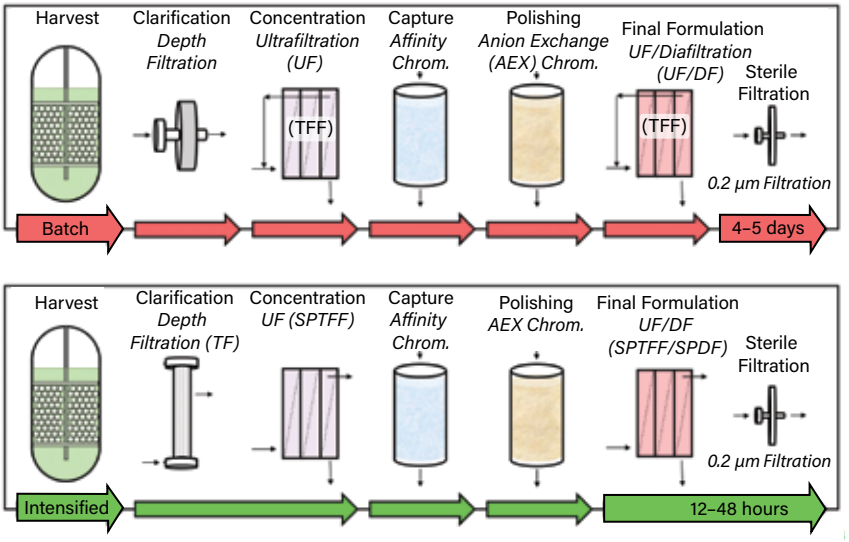
One major challenge in AAV manufacturing is the high clinical dosage requirement, typically 1,000–10,000 times greater than the titer of AAV material from the bioreactor. Therefore, concentrating the dilute virus is critical to the downstream process. Product concentration is typically achieved using tangential flow filtration (TFF), wherein retained AAV particles flow tangential to the membrane to mitigate surface fouling. Current processes use batch TFF, in which the product stream is recirculated through the membrane module while buffer and impurities are removed in the permeate. Batch TFF requires large equipment footprints and long operating times, and the recirculation of the shear-sensitive AAV vectors can cause capsid aggregation and/or fragmentation, degrading the quality of the final therapeutic.

Through an ongoing industry-academic collaboration facilitated by the Membrane Applications Science and Technology (MAST) Center, researchers at Penn State, the Univ. of Arkansas, and Spark Therapeutics have developed a single-pass tangential flow filtration (SPTFF) process for purification and concentration of AAV clarified cell lysates (CCL). The MAST Center is an Industry-University Cooperative Research Center funded by the U.S. National Science Foundation (NSF). Unlike traditional TFF, SPTFF concentrates AAV vectors along the length of an appropriately staged membrane device in a single pass without any recycling. The high concentration factor is achieved by optimizing the flow and pressure distribution within the staged device to minimize membrane fouling, which can occur due to the accumulation of AAV material at the membrane surface. In addition to the reduced process shear, SPTFF can also increase productivity by enhancing the performance of subsequent capture chromatography step(s).

An initial proof-of-concept SPTFF pilot-scale system successfully concentrated 10 L of AAV CCL down to 1 L. The process was completed while maintaining a 99% AAV yield and achieving a 37% reduction in host cell proteins. Linkage of SPTFF with the subsequent affinity chromatography step significantly improved overall productivity compared to a conventional batch process. This included a four-fold reduction in the affinity chromatography step's operating time, which is used to remove key impurities.

Aside from its utility in intensifying existing batch processes, SPTFF may also serve as a key component in the development of a fully continuous AAV downstream production platform.

This success highlights the value of industry-academic partnerships in driving bioprocess innovation. "This project provided the first demonstration of SPTFF to concentrate and purify AAV CCL, marking a significant step towards the development of intensified and/or continuous platform processes for gene therapy manufacturing," says Christopher Yehl, Senior Scientist at Spark Therapeutics and advisory board member with the MAST Center. CEP



▲ A novel single-pass tangential flow filtration (SPTFF) system is capable of concentrating and purifying adeno-associated viral (AAV) clarified cell lysate (CCL), enabling a transition from traditional batch operations (top) to intensified (bottom) and/or continuous bioprocesses.

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