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Use of SPTFF in Continuous Downstream Manufacturing of Adeno-Associated Viruses (AAV)

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ABSTRACT

In recent years, pre-clinical and clinical development in the gene therapy industry has been rapidly growing. To meet the industry's requirement for large quantities of GMP-compliant therapeutic viral vector, there is a need for high-efficiency equipment and consumables. A typical downstream process for AAV manufacturing involves a combination of unit operations including clarification of crude harvest, chromatography, concentration, diafiltration and sterile filtration.

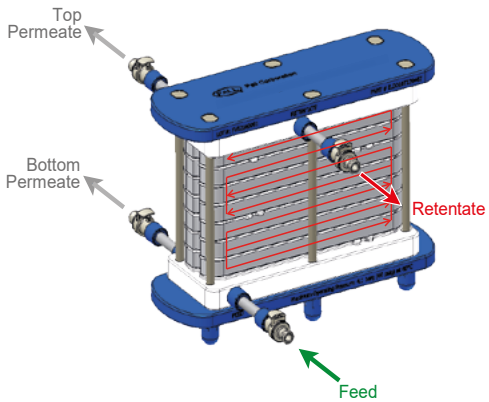
Some challenges in AAV processing include processing-time and shear sensitivity of the product and safety concerns of the product. Adding tangential flow filtration (TFF), membranes into a process can reduce working volumes and application of single-use consumables can mitigate safety concerns.

Replacing traditional recirculating TFF with single-pass TFF (SPTFF) technologies has the potential to reduce shear exposure, reduce processing time by integrating unit operations before and/or after, and improve process yields. In this work, we implemented Pall's innovative Cadence® SPTFF technology for in-line concentration to optimize an AAV downstream process. An SPTFF device was connected to an upstream depth filtration assembly with a small break tank. The post-SPTFF-concentrated viral vector stream was continuously pumped through a sterile filter. This work demonstrates the use of an integrated, continuous SPTFF operation in an AAV process that achieves 40% reduction in processing time while maintaining a 96% yield.

INTRODUCTION TO SPTFF

- SPTFF consists of multiple stacks of Pall's Centramate™ TFF cassettes arranged in series for the concentration of feedstock to volumetric concentration factors (VCF) of 4 to 30X in a single pass.
- Advantages includes elimination of recirculation loop, 5-10X lower feed flow rate, smaller line size, no large accumulation tanks, lower working volume for higher recovery. Figure 1 provides an example of the flow path of an SPTFF device.

Figure 1
Cadence SPTFF module with cassettes



MATERIALS & METHODS

Feedstock

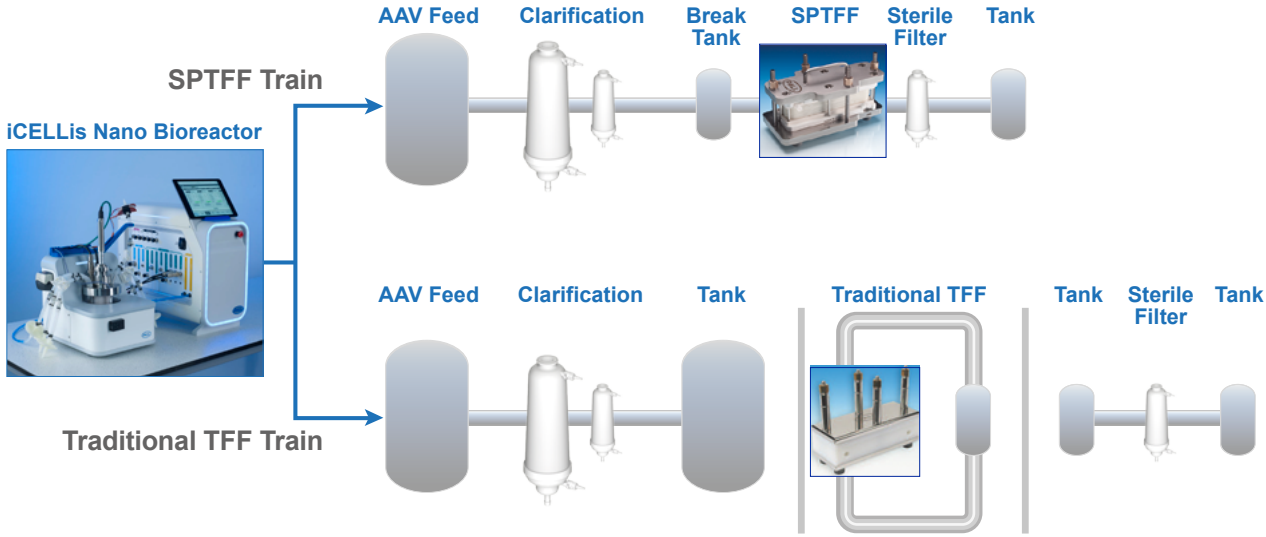
- AAV5 produced in HEK293 cells using Pall's iCELLis® Nano bioreactor.
- On the day of experiment, cells were lysed, and the lysate was combined with bioreactor supernatant and washed.

Setup

- Two process trains were assembled consisting of three-unit operations: clarification, TFF concentration and sterile filtration.
- Figure 2 provides a diagram showing the two process flow paths.
- SPTFF: A Cadence single-pass TFF modular kit, consisting of manifold plates, flow directional gaskets, Pall's Omega™ 100 kDa polyethersulfone (PES) cassettes and holder was used for this study. The SPTFF device was assembled in a 4-stage configuration with parallel cassette arrangements of 8:6:2:1. A peristaltic pump was used to provide feed flow and pressure from a small break tank. The concentrated viral vector was pumped directly from the SPTFF device through a sterile filter.
- Traditional TFF: An Omega 100 kDa PES cassette and holder was attached to a Spectrum♦ KR2i TFF system set up to run as a standard batch operation.

Figure 2

Experimental setup using SPTFF and traditional TFF for ultrafiltration application



Operation

- An AAV virus feed was divided into two equal pools of 10 L and processed through two separate downstream trains; a continuous process train, using SPTFF and a batchprocess train using traditional TFF.
- For each experiment, PBS buffer was used to pre-flush the systems and filters.
- For each experiment, clarification was performed using cellulose-based depth filter in-line with membrane sterilizing-grade sized to process at 200 LMH.
- SPTFF was scaled to process 200 LMH flow from the clarification filter. The flow rate of the concentrate stream from the SPTFF was controlled to achieve steady operation through sterile filtration.
- For each experiment, a targeted viral vector concentration of 10X was achieved before chromatography.
- Samples of the post-TFF sterile filtrate were collected to calculate yield.

Analysis

- Sample aliquots were collected before and post processing were to perform ddPCR for AAV5 yield analysis.

RESULTS & DISCUSSION

- The targeted concentration factor of 10X was achieved for both the SPTFF and the traditional TFF methods.
- The feed flow rate for the SPTFF was significantly lower (~5X less) than the traditional TFF method, allowing for lower shear.
- Figure 3 presents the SPTFF process data showing a continuous permeate flow rate at the targeted constant concentration factor for the duration of the run. There was also a steady inlet pressure rise observed with this flow path configuration.
- Figure 4 presents the process data for the traditional batch TFF concentration. The data shows a typical decline in permeate flow over the course of the run with a minor increase in transmembrane pressure (TMP).
- The total process time was significantly shorter for the SPTFF as shown in Table 1.

Figure 3

Experimental setup using SPTFF for ultrafiltration application

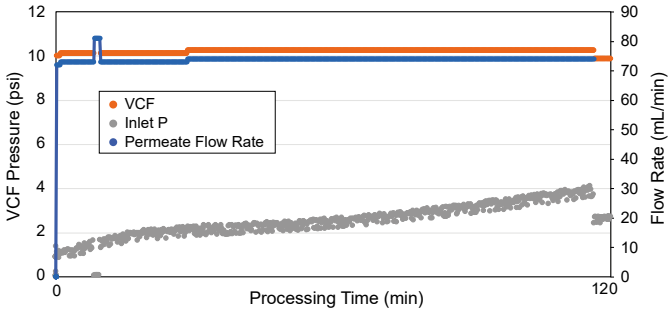


Figure 4

Experimental setup using traditional TFF for ultrafiltration application

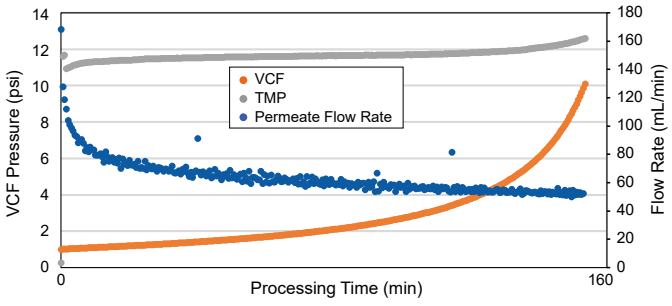


Table 1

SPTFF and traditional TFF process time

	Clarification Time (min)	Concentration Time (min)	Sterile Filtration Time (min)	Total Process Time (min)
SPTFF	Continuous process			190
Traditional TFF	153	159	7	319

- ddPCR analysis of final concentrate samples for both the SPTFF and the traditional TFF shows similar yields for both methods.

Table 2

SPTFF and traditional TFF ddPCR yields

	Clarification Yield (%)	Concentration Yield (%)	Sterile Filtration Yield (%)	Total Yield (%)
SPTFF	Continuous process			96.1 ± 9.3
Traditional TFF	96.2	102	98	96.2 ± 6.3

CONCLUSION

- **Both the SPTFF and traditional batch TFF process methods concentrated an AAV viral vector feed to a 10X concentration factor with acceptable yields.**
- **The SPTFF process method was able to achieve the targeted concentration level at a lower feed flow rate in a shorter process time. This could result in higher yields for more sensitive feed streams.**

Future Work

- **Examination of the SPTFF flow path and viral loading to reduce the pressure rise during operation and membrane fouling.**
- **Comparison of both SPTFF and traditional TFF on a more shear-sensitive feed.**

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