Towards Continuous Manufacturing for High-Dose, Cost-efficient rAAV Therapies

CATAPULT Cell and Gene Therapy

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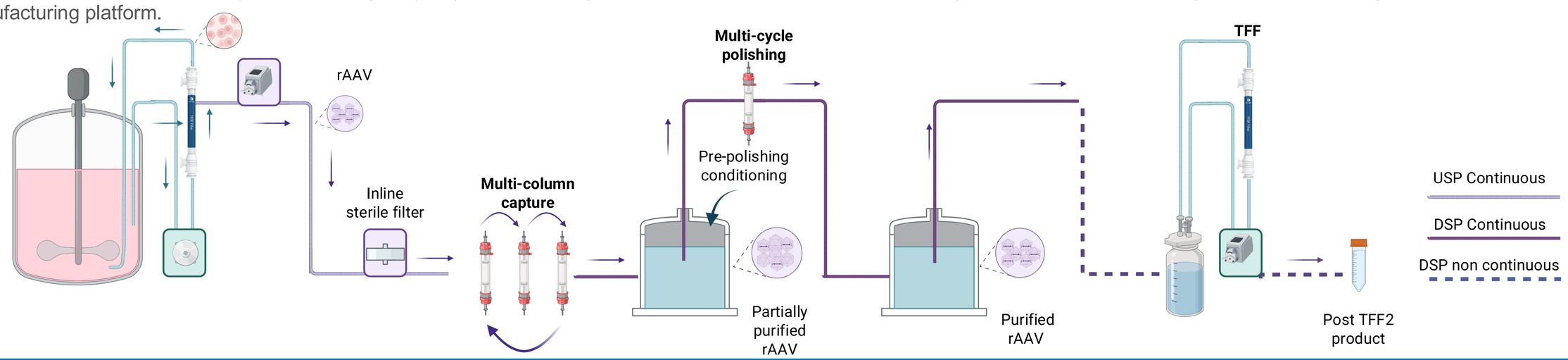
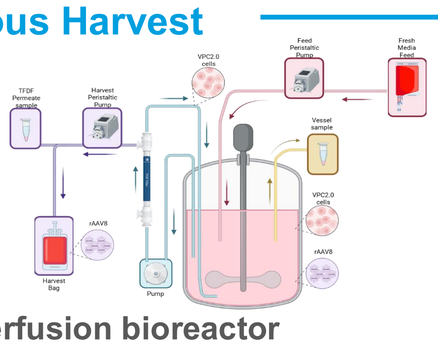
Introduction and Process Overview

Continuous manufacturing addresses key challenges in recombinant adeno-associated virus (rAAV) production by improving scalability, yield, and process efficiency. This work presents a newly developed upstream (USP) perfusion platform with continuous harvest, enhanced feeding strategies, and improved transient transfection to enhance productivity. In parallel, downstream process (DSP) innovations focus on enhancing purity, scalability, and cost-effectiveness through continuous capture chromatography, DNA impurity clearance, and implementation of process analytical technologies (PAT) for TFF2. Together, these developments represent key steps toward establishing a seamless, next-generation continuous rAAV manufacturing platform.



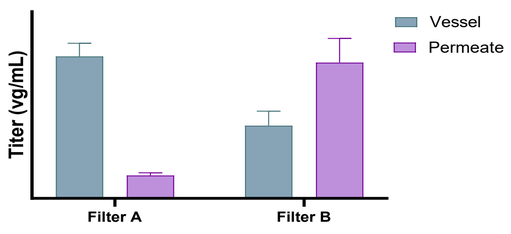
Challenge 1: Continuous Harvest

In this system, rAAV vectors are continuously produced and secreted into the media post-transfection, supported by a perfusion-based process as long as cells remain viable and productive.



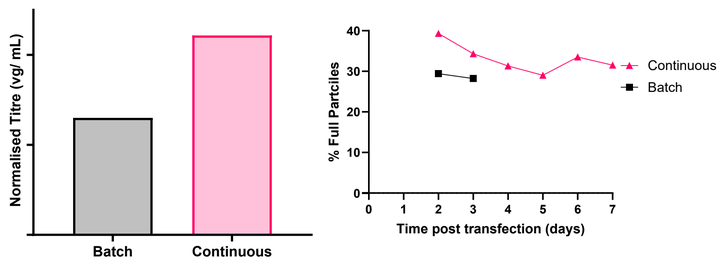
Filter Selection for secreted rAAV in 2 L perfusion bioreactor

- Assessed membrane transmission by quantifying viral genomes (VG) in vessel and permeate fractions
- Filter B increased rAAV released from the vessel to the permeate achieving continuous harvest



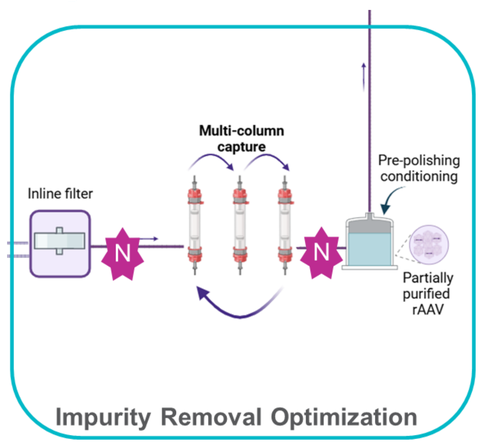
2x Increase VG titre in 2 L perfusion bioreactor using Filter B

- Standard cell density transfection was used to evaluate Filter B performance.
- Continuous perfusion increased total accumulated rAAV yield compared to standard batch processing.
- Product quality was maintained over a 7-day perfusion period.

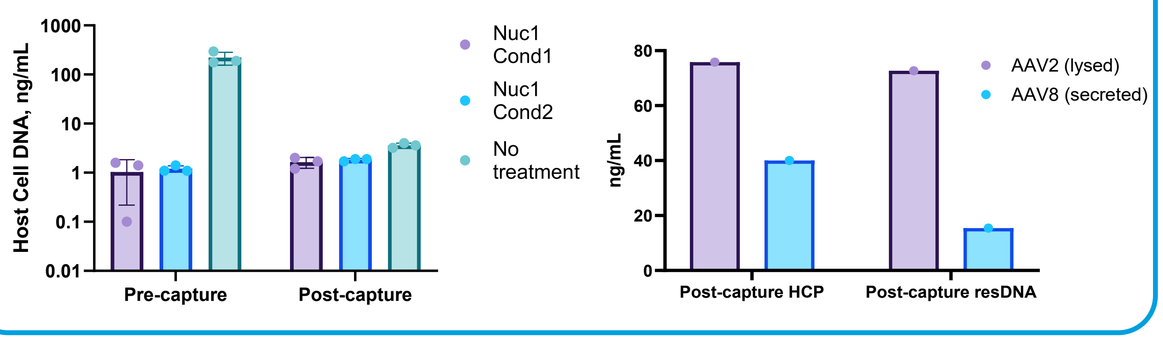


Challenge 2: DNA impurity Removal

Impurity removal remains a challenge in downstream processing (DSP) for both batch and semi-continuous rAAV production, given strict regulatory limits on residual DNA. In semi-continuous platforms, where rAAV is secreted during continuous upstream processing, targeted strategies have been developed to efficiently remove DNA and other impurities from harvested material, supporting product quality and regulatory compliance.

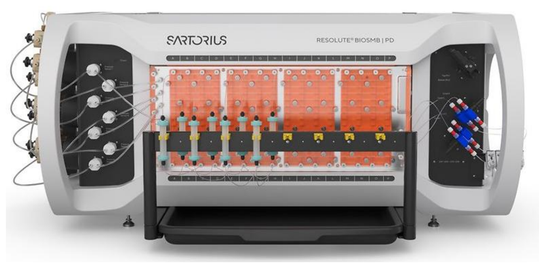


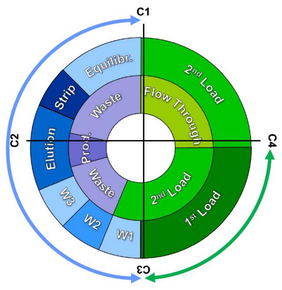
- ❑ **Lysis and nuclease treatment post-harvest** were deemed **unnecessary**, as their omission did not impact DNA impurity levels.
- ❑ Using **secreted material without nuclease resulted in lower impurity levels** compared to lysed, nuclease-treated material.



Challenge 3: Continuous Capture Chromatography

Development of a single-column method to be followed by implementation of a multi-column chromatography approach, which benefits include improved automation and process control, increased productivity and cost efficiency through better resin utilization.

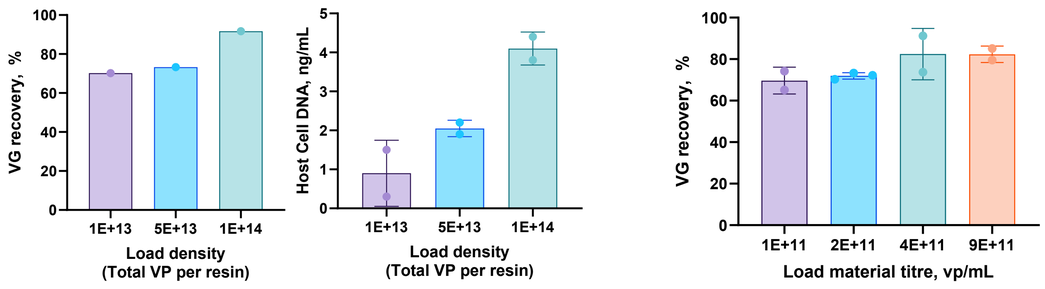




The chronogram helps to understand and visualise column cycling behaviour and shows how productivity is maximised by ensuring no overlaps and idle columns. As one column loads, the other is regenerated to accept further material.

Load Density Screening: improved genomic titre (viral genomes, VG) recovery at the cost of increased residual impurities.

Impact of Titre Variability at Fixed Load Density: stable recovery indicating limited influence on rAAV yield

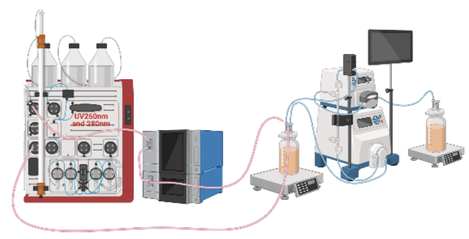


A controlled and normalized loading strategy is essential to ensure robust and consistent performance of multi-column capture systems in continuous processing.

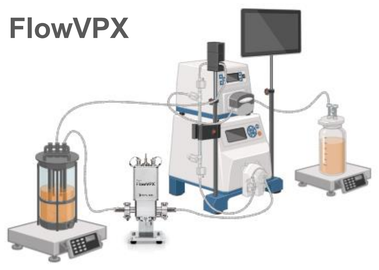
Challenge 4: PAT for TFF2

Two **Process Analytical Technology (PAT) tools** were evaluated for monitoring the tangential flow filtration (TFF) step: MALS (Wyatt) and FlowVPX (Repligen). Both approaches showed strong potential for enabling titre estimation, real-time process control, and non-invasive sampling, **supporting enhanced monitoring and automation of the TFF process.**

Multi-Angle Light Scattering (MALS)



FlowVPX



Conclusion

Semi-continuous manufacturing of recombinant AAV (rAAV) is emerging as **a scalable and efficient platform** that integrates intensified upstream and downstream processes into a unified workflow. This holistic approach has the potential to significantly **enhance product yield and quality** while **boosting overall productivity and reducing manufacturing costs.** By incorporating process analytical technologies (PAT), digital tools, and automation, we aim to further optimize performance and enable robust, therapeutic-grade rAAV production. Future work will focus on fully connecting continuous upstream with continuous downstream operations to **establish a seamless, next-generation rAAV bioprocessing platform.**

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