Therapeutic Viral Vectors: Manufacturing, Challenges and Platform-Based Approach

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Introduction

Emerging therapeutics such as mRNA vaccines and viral vector-based gene therapies are more complex than established modalities such as monoclonal antibodies (mAbs) and recombinant proteins. The challenge arises in how to produce these new therapies at scale and cost effectively. The higher complexity of viral vectors compared to mAbs creates bioprocess challenges; for example, with viral vectors, production and purification scientists must deal with complex quaternary structures for which the biophysics and biochemistry are still not well-characterized. For many indications, a 100-fold increase in overall process yield of potent vectors is required, which must come from increased titers during upstream production and recovery during downstream processes without compromising on critical quality attributes. A large shift in technological innovations must take place to enable the industrialization of new modalities' manufacturing with a reduced cost in mind.

Collaboration Objective

To deliver platform-based approach solutions for maximizing AAV and Lentivirus manufacturing yield using scalable and low shear technologies that enable cost-effective commercialization of these advanced therapies.

Advanced AAV/LV Manufacturing Workflow

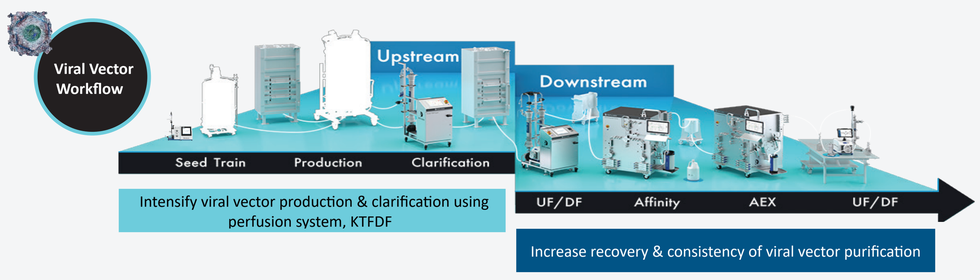


Figure 1: Advanced viral vector end-to-end manufacturing process.

Viral Vector Industrial Upstream Perfusion Platform

Intensify viral vector production by integrating perfusion & Clarification Platform: KrosFlo TFDF

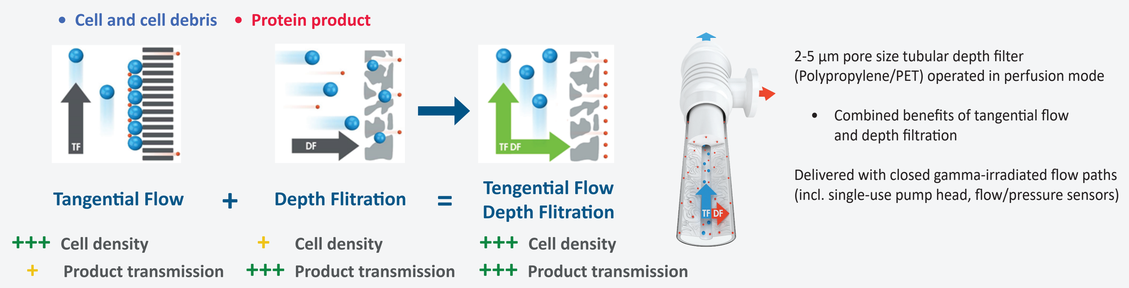


Figure 2: 2-5 µm pore size tubular depth filter (Polypropylene/PET) operated in TFF mode. Combined benefits of tangential flow and depth filtration.

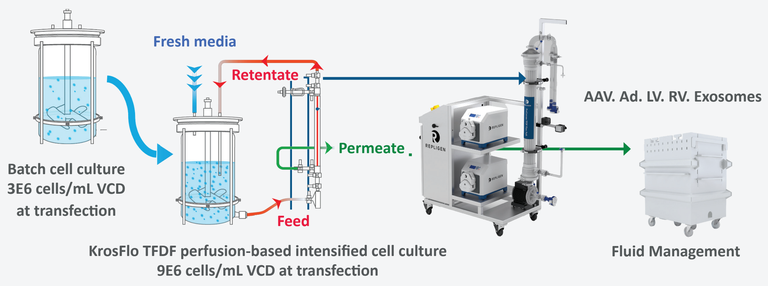


Figure 3: KrosFlo TFDF-based Perfusion System enabling high cell density at time of transfection, high specific productivity and viral vector transmission through the TFDF pore sizes.

Viral Vector Industrial Downstream Capture Chromatography Scale-Up Platform

Intensify viral vector recovery by using the advanced KRM Platform: Reduce hold-up volume and minimize cross-contamination to increase recovery

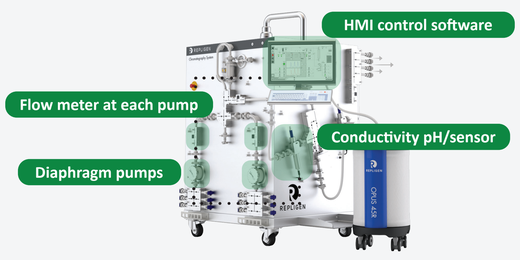


Figure 4: KRM Chromatography System.

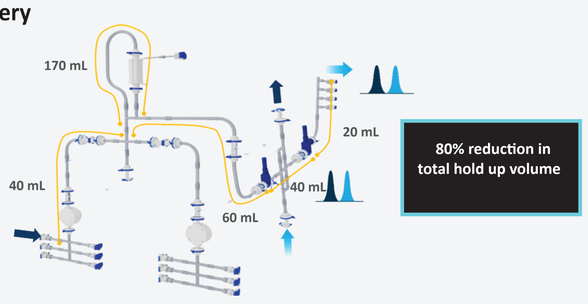


Figure 5: KRM pre-assembled flow paths with low hold-up volumes.

Figure 6: Flow pattern comparison of traditional barb fittings (left) and over-molded low shear connections (right).

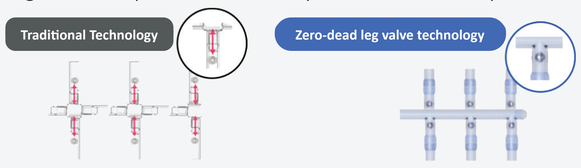


Figure 7: Comparison of traditional pinch valve assembly design (left) and the overmolded valve manifold design (right).

Upstream Intensification Using KrosFlo® TFDF® Perfusion System

Case studies: AAV Intensification

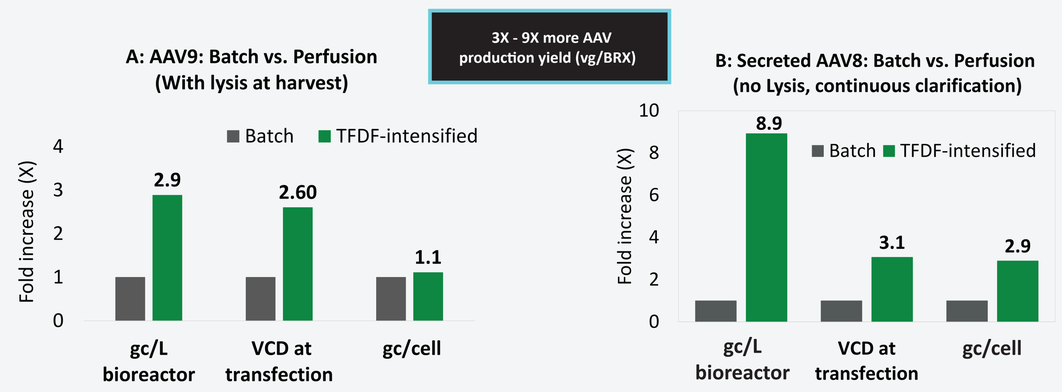


Figure 8: Intensify cell density at time of transfection to 9e6 VCD/mL using TFDF Perfusion System. The cells were transfected on Day 3 using TransIT-VirusGEN® AAV Kit (Mirus Bio). In Figure 8a the cells were in lysis prior to harvest and in Figure 8b the virus was secreted into the spent media and separated continuously using the TFDF Perfusion System. 2.9 X more AAV9 production yield (vg/BRX) and 8.9 X more AAV8 production yield (vg/BRX) in Figure 8a and 8b respectively.

Case studies: LV Intensification

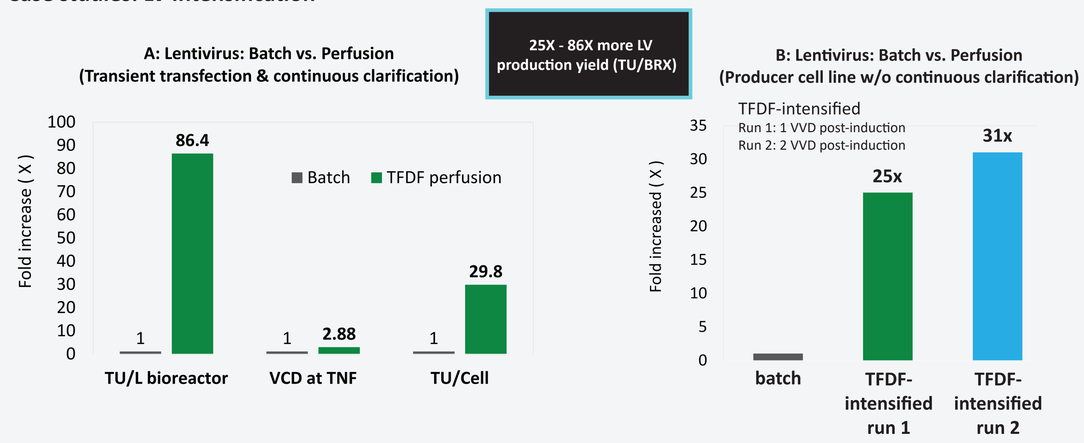
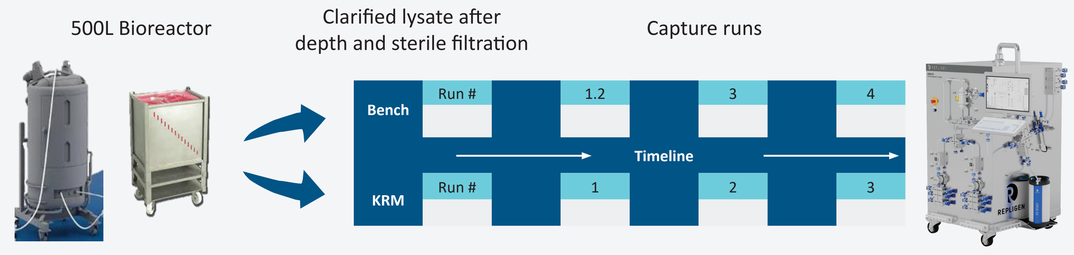


Figure 9: Intensify cell density at time of transfection to 9e6 VCD/mL using TFDF Perfusion System. In Figure 9a the virus was separated continuously from the cell culture into the spent media using the TFDF Perfusion System. 86 X more LV production yield (TU/BRX) and 25 X more LV production yield (TU/BRX) in Figure 9a and 9b respectively.

Downstream Intensification Using KRM™ Chromatography System

Case study: Scalability and reproducibility of the AAV9 capture step using KRM Chromatographic System



Run #	Bench top 1	Bench top 2	Bench top 3	Bench top 4	KRM 10 1	KRM 10 2	KRM 10 3
Load volume [1]	1			0.6	50	167	
Flow rate [mL/min]	2.09				214		
Peak volume [L]	0.009	0.009	0.01	0.008	170	198	190
Peak area ratio	2.1				7.3	8.6	8.3

Figure 10: Evaluation of KRM Chromatography System as process development and manufacturing platform. Scalability from 1 L bench top system to KRM 10 System and reproducibility performance of the scale up system.

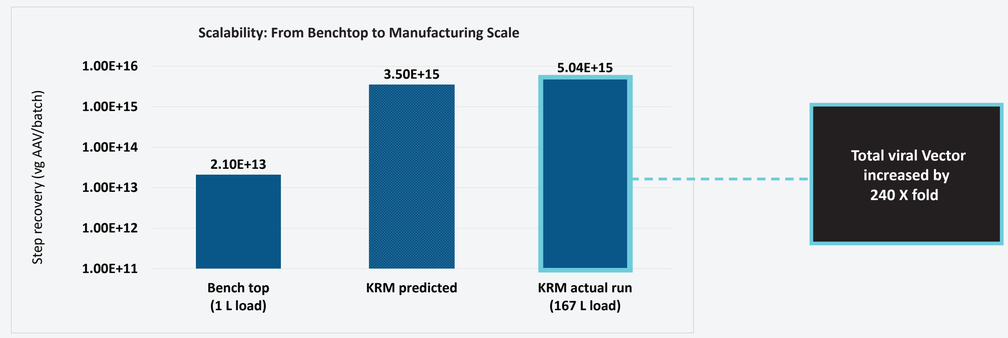


Figure 11: The scalability of the AAV capture step from the benchtop to the manufacturing scale using KRM Chromatography Systems was analyzed by comparing process performance and product quality at two scales. There was no increase of endotoxins, particle aggregations, or residual non-product related impurities such as HCP (data not shown).

Conclusions

KrosFlo TFDF-based perfusion viral vector intensification:

- 2.9X AAV9 and 9X AAV8 increase production yield per batch
- 25X -86X LV increase production yield per batch
- Continuous perfusion is critical to intensified AAVs and LVs production through enhanced cell specific productivity
- No limitation for virus production from nutrient deprivation and/or accumulation of inhibitory metabolites

Downstream collaborative study between Forge Biologics and Repligen successfully verified the scale-up of an AAV capture purification step to manufacturing scale:

- By maintaining the quality attributes such as purity and even improving process recovery while maintaining process parameters
- High recover yield (136%) of AAV9 was achieved at large scale
- Due to the gentle fluid management, low hold-up, and accurate pump performances of the KRM 10 System, higher recovery and high consistency between the control benchtop and large-scale runs were observed

Reference

1. Yen Tran et all, 2022 , https://www.frontiersin.org/articles/10.3389/fbioe.2022.887716/full

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