Filtration Insights in Gene Therapy Processing

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ABSTRACT

The gene therapy industry is growing rapidly, with over 20 therapies now approved worldwide and hundreds more in clinical trials. As the field reaches a wider range of indications with larger patient populations, some added focus must be placed on developing more efficient, scalable manufacturing processes.

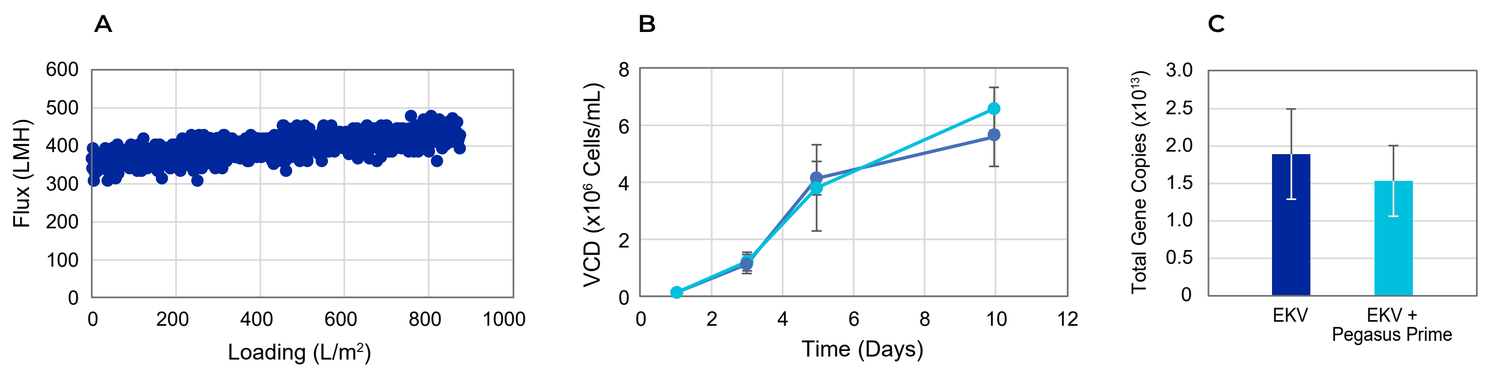
Early gene therapy manufacturing has largely borrowed technologies developed for recombinant protein processing. This has generally translated well, but there is still significant room for improving yield and product quality. In this work, we focus on filtration technologies including depth filtration, tangential flow filtration, sterile filtration, and viral filtration. We will discuss how well each has translated into processing of adeno-associated virus (AAV) and lentivirus, two of the largest classes of gene therapies, and highlight areas for improvement.

SUSPENSION CELL CULTURE & ADHERENT CELL CULTURE

VIRAL RISK MITIGATION

Adventitious viral clearance can be especially challenging for gene therapy processes, therefore increasing the importance of preventing adventitious virus introduction through raw materials. The literature has shown CHO cell culture media can be processed through 20 nm virus filters with no significant impact on culture performance[1]. Our data suggests this strategy can translate to gene therapy. In an internal study, we observed no fouling on a Pegasus™ Prime virus removal filter challenged with HEK293 media, and no measurable impact to cell culture health or virus production.

Figure 1
Nanofiltration of cell culture media used in AAV production. *A batch of DMEM + NEAA + Glutamax♦ media was split and run through either a Supor® EKV sterile filter or a Pegasus Prime virus filter + Supor EKV sterile filter.* ***A.*** *Flux vs. loading on a Pegasus Prime filter run at a constant dP of 2.1 bar.* ***B & C:*** *HEK293 cells producing recombinant AAV5 were grown in iCELLis® Nano bioreactors with each media batch. A comparison of viable cell density (VCD) and AAV titer is shown.*



CLARIFICATION

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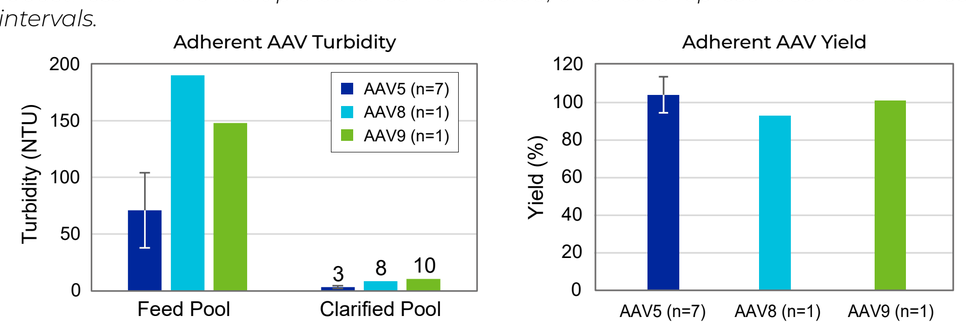
Depth filter and sterile filter process robustness, scalability, and process economics have all translated well into gene therapy processing. Combining this with the important benefit of being single-use makes direct filtration an attractive option for gene therapy clarification.

Comparing today's AAV processes to recombinant protein processes, the significantly lower cell densities (>5x lower) have been more impactful than the lower viabilities (AAV-producing cells are often lysed to release more product). This has translated to higher overall volumetric capacities on depth and sterile filters. In our work, AAV yields typically exceed 90%[2].

Lentivirus clarification has revealed more challenges with yield, and we typically see a performance trade-off between cellulose-based depth filters (high capacity, low yield) and more inert synthetic filters (e.g., polypropylene, glass fiber; low capacity, high yield).

Figure 2
Turbidity reduction and AAV yield. *AAV5, 8, and 9 crude harvests from adherent culture were clarified using bench- and pilot-scale PDK11 depth filters (2–20 µm) + Supor EKV sterile filters (0.2 µm). Turbidity reduction and step yield data shows strong process robustness. Where multiple batches were tested, error bars represent 95% confidence intervals.*



TANGENTIAL FLOW FILTRATION

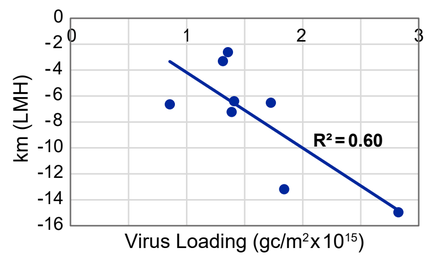
CONCENTRATION / DIAFILTRATION

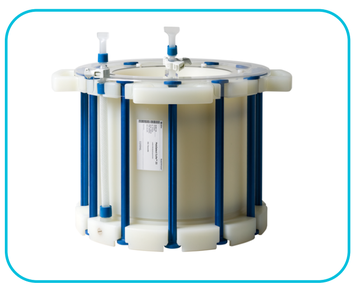
Tangential flow filtration (TFF) is an economical and scalable technology for concentration and buffer exchange and is now regularly used in gene therapy processing. With limited process development, we observed an average 89% step yield (n=8) at an intermediate AAV5 concentration step using Pall T-series cassettes with Omega™ (polyethersulfone [PES]) 100 kDa membrane, demonstrating the viability of flat-sheet TFF for AAV processing. Consistent with TFF used in early mAb/protein process steps (pre-affinity), we also observed some membrane fouling which should be considered during development (Figure 3).

Notable differences for gene therapy processing include a preference for closed systems, less process restriction caused by drug substance viscosity, and higher product sensitivity to process conditions (e.g., time, temperature, shear).

The question of product sensitivity remains open for the field, prompting us to evaluate a low-shear single-pass TFF process. We did not observe any benefit to pool turbidity or product yield from the reduced shear exposure. However, as expected, connecting the adjacent processes into a continuous process can reduce overall processing time (Figure 4).

Figure 3
TFF fouling from a clarified AAV5 feed. *Eight separate batches of AAV5 were clarified then loaded onto Omega (PES) 100 kDa TFF cassettes at ~190 L/m². Each pool was concentrated to a target 10x concentration. Flux vs. ln(C) plots were created and mass transfer coefficients were taken from the slope of a linear fit. Mass transfer coefficients from each batch are plotted here against virus loading on the TFF cassettes. The downward trend with increased loading is likely indicative of membrane fouling.*



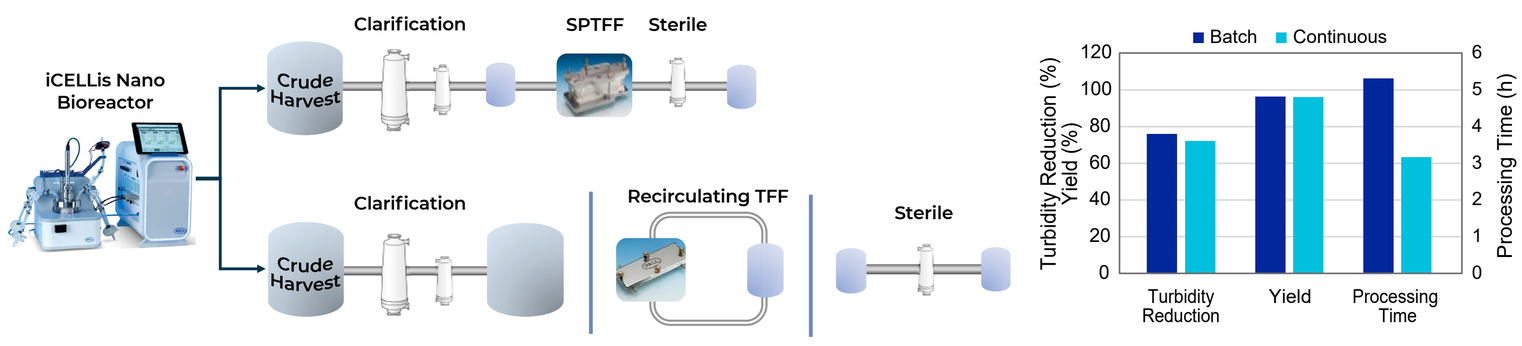


AFFINITY CHROMATOGRAPHY

IEX CHROMATOGRAPHY

CONCENTRATION / DIAFILTRATION

Figure 4
Continuous vs. batch TFF of a clarified AAV5 pool. *An AAV5 crude harvest pool was split and run through two processes in parallel. The first used Omega (PES) 100 kDa TFF cassettes in Pall's Modular SPTFF kit to connect the clarification, concentration, and sterile filtration into one continuous process. The second used the same consumables setup in a more traditional set of batch processes. Both achieved a 10x volumetric concentration. Data compares turbidity reduction, yield, and processing time over the two processes.*



STERILE FILTRATION

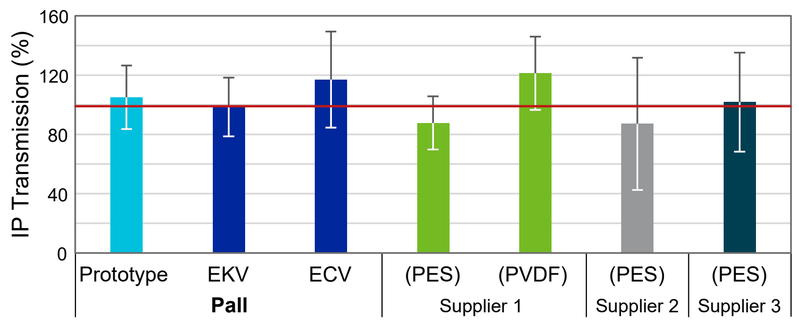
The ICH Q11 regulatory guidance requires an adequate control strategy to ensure sterility of final drug substance pool. In the recombinant protein industry, this is achieved by filtering final drug substance through 0.2 µm validated sterilizing-grade filters.

Despite the smaller difference in size between the membrane pores and a large vector such as lentivirus, we have found consistently high transmission of the product, with no substantial benefit between different sterile filters on the market (Figure 5).

One key difference has been the filter capacity, which for lentivirus feeds is often <100 L/m². From our research, this appears to be linked to higher turbidities in the UFDF pool (>100 NTU).

Another key difference is lower viscosities seen in gene therapy drug substance pools. This has allowed for significantly higher fluxes with little impact seen on capacity or yield.

Figure 5
Lentivirus sterile filter transmission. *Lentivirus UFDF pools were split and run through a range of 0.2 µm sterilizing grade filters in parallel at 150 LMH until reaching a terminal pressure of 0.7 bar. Infectious particle transmission is shown. Error bars represent 95% confidence intervals over three batches.*



CONCENTRATION / DIAFILTRATION

VIRAL FILTRATION

FINAL FILTRATION

The recombinant protein industry uses a robust set of orthogonal viral clearance steps to ensure removal of adventitious viruses by inactivation, charge, and size. Viral clearance for gene therapy products has been more limited to date, and has mostly focused on preventing adventitious virus introduction, and charge-based separation.

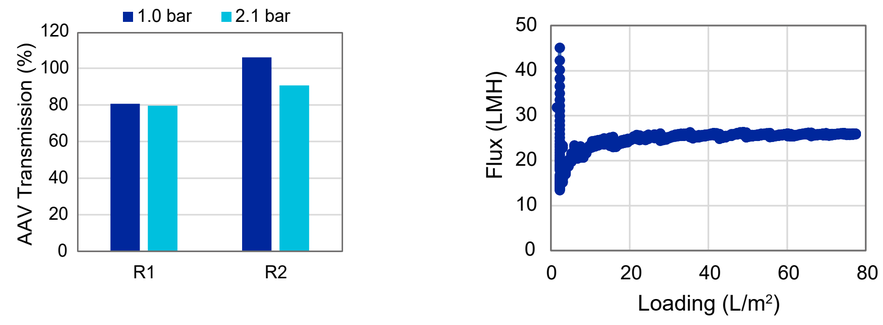
Size-based separation is particularly challenging as the product is often similar in size to the contaminant.

However, we have shown that AAV can achieve ~90% transmission through Ultipor® DV50 membranes validated for >6-log removal of viruses 50 nm and larger, which could add to the overall control strategy.

References

1. *Liu S, Carroll M, Iverson R, Valera C, Vennari J, Turco K, et al. Development and qualification of a novel virus removal filter for cell culture applications. Biotechnol Prog. 2000;16(3):425–34.*
2. *Chinnawar R, Marchand N. Clarification of recombinant adeno-associated virus (rAAV) & lentivirus from adherent culture. Cell Gene Ther Insights. 2022;8(3):483–93.*

Figure 6
Virus filtration of AAV5 drug substance. *AAV5 drug substance pools were split and run through 13 mm discs of Ultipor VF Grade DV50 membranes at 1.0 and 2.1 bar. AAV transmission from two replicate batches is shown alongside flux data from a representative run at 2.1 bar.*



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