Simplifying Progress

Clear It Up! – Alluvial Filtration for Efficient Clarification of Suspension HEK293 Process Harvest in AAV Production

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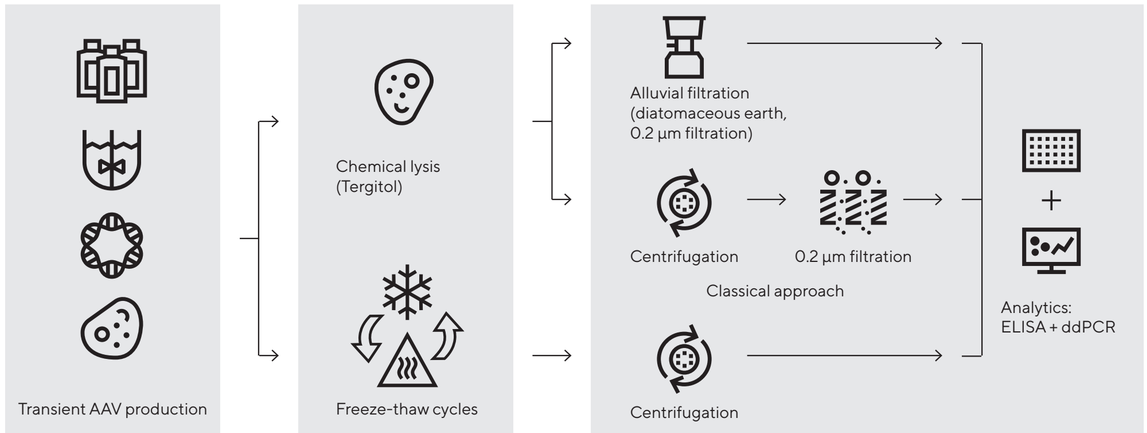
Introduction

The rapidly increasing demand for adeno-associated virus (AAV) vectors necessitates novel strategies for large-scale manufacturing. While AAV production in suspension culture allows for easy upscaling, downstream processing of large culture volumes remains challenging. Due to serotype-dependent intracellular localization of AAVs, release of viral capsids from the producer cell is mandatory and can be accomplished e.g. by multiple freeze-thaw cycles or chemical lysis. This step is classically followed by centrifugation and filtration and further downstream processing. However, such an approach is not feasible for large scale AAV production, where centrifugation steps are time consuming and challenging to scale, while filters easily become clogged. In this work, harvest clarification and removal of cellular debris subsequent to chemical cell disruption was realized through alluvial filtration as an alternative method.

Methods

Commercially available HEK293 suspension cells were cultivated in chemically-defined culture medium HEK ViP NB and feed HEK FS (both Sartorius Xell GmbH) in 2 L bioreactor scale. FectoVir-AAV (Polyplus) was used for transient transfection with a two-plasmid system for AAV2, AAV5 and AAV8 (Plasmid Factory) with GFP as GOI. For cell lysis a Tergitol TMN-100x (Sigma Aldrich)-based lysis buffer containing benzonase was used, and culture was continuously stirred (1 h, 37 °C). For further downstream processing, the harvest was split in half for either clarification by centrifugation (classical approach), or by the addition of diatomaceous earth (DE; Sartoclear Dynamics® Lab Filter Aid, Sartorius AG) (alluvial filtration approach), both followed by 0.22 µm PES filtration using the Sartolab® Multistation (Sartorius). Efficiency was analyzed by turbidity measurement, ddPCR (BioRad) and ELISA (Progen) for genomic and capsid titer, respectively.

Figure 1: *Flow Chart Showing the Harvest Clarification Processes After AAV Production*

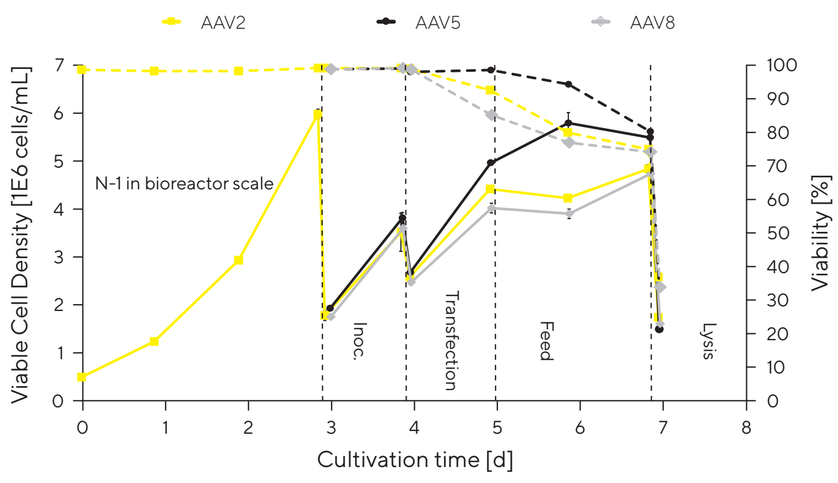


Results

AAV Production in 2 L Bioreactor Scale in HEK293 Suspension Cells

For comparison of different methods for clarification of culture harvest after AAV production and cell lysis, AAV serotypes 2, 5 and 8 were produced in 2 L bioreactor scale. The culture profiles were very similar between the serotypes. Successful cell disruption via chemical lysis was monitored by a decrease in viability and viable cell density.

Figure 2:

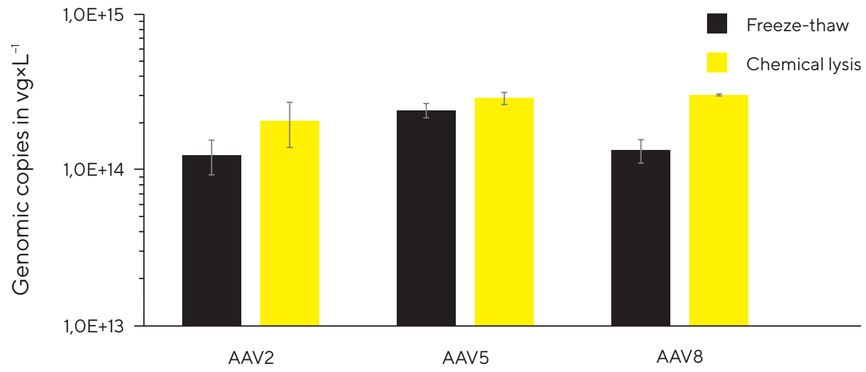


Note: Viable cell density and viability of HEK293 cells during cultivation in a 2 L bioreactor for N-1 and production phase. Cells were seeded at a cell density of 3×10^5 cells/mL on day zero in a preculture/N-1 bioreactor. On day three, AAV-2, AAV-5 and AAV-8 production processes were inoculated at a cell density of 2×10^5 cells/mL. Transient transfection was performed 24 hrs after seeding with a 2-plasmid system, with 1 µg/mL DNA per 1×10^6 cells and FectoVIR-AAV as a transfection reagent. For cell lysis 72 hr after transfection, the culture was continuously stirred (1h, 37 °C) after addition of a Tergitol TMN-100x-based lysis buffer containing benzonase.

Comparison of Freeze-Thaw vs. Chemical Lysis

For AAV release from HEK293 producer cells, either three freeze-thaw cycles of pelleted cells right before harvest or chemical lysis in the bioreactor at harvest with a Tergitol TMN-100x solution was performed. Irrespective of the serotype, genomic titers revealed that chemical lysis was equal or superior to lysis by freeze-thaw, while also being the method that is scalable for larger production.

Figure 3: *Genomic Titers Post Lysis/Pre Filtration*

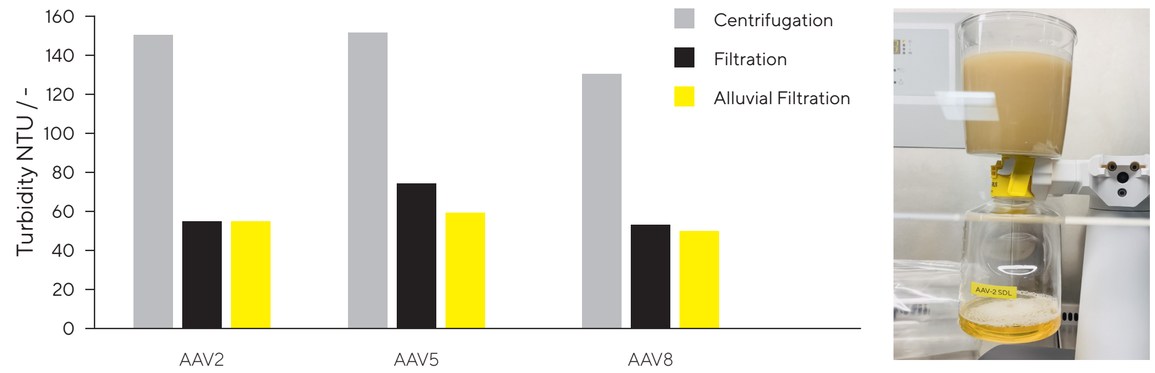


Note: AAV genomic titer after freeze-thaw (black) or chemical (yellow) cell lysis. For freeze-thaw titers, the sum of the AAVs released in the culture supernatant and those released from the cells by cell lysis is shown. Chemical lysis was at least as efficient as the classic freeze-thaw method.

Centrifugation-Free Clarification of Lysed Cell Culture Harvest

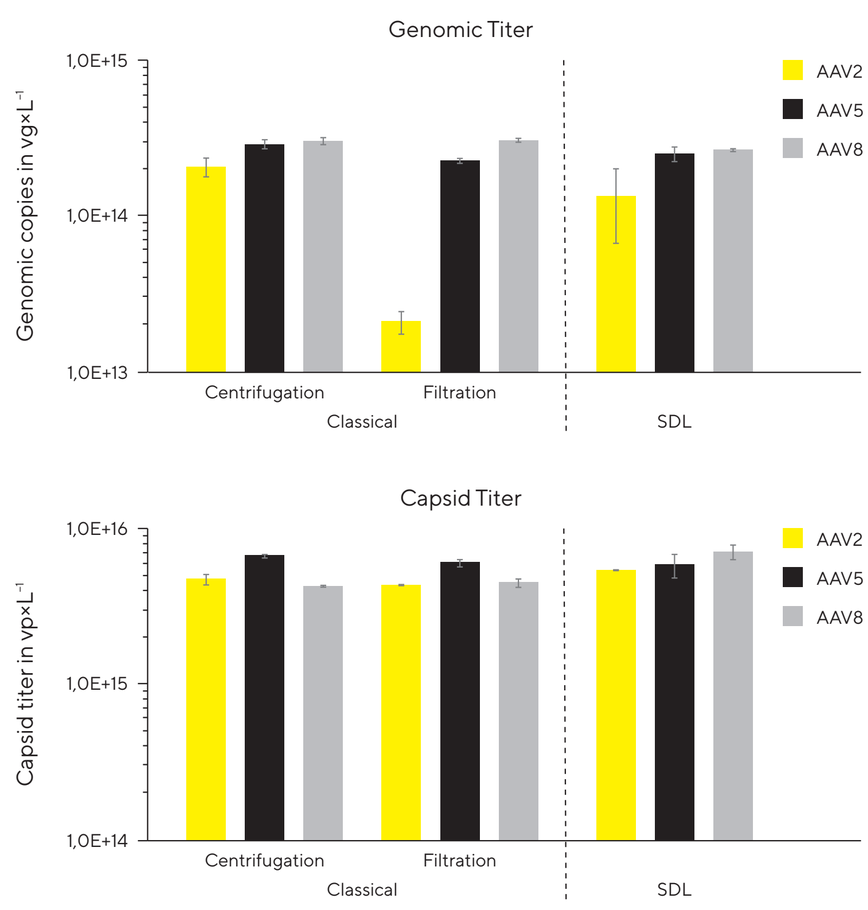
Harvested culture after cell lysis was split in half for either clarification by centrifugation or by the addition of diatomaceous earth, both followed by 0.2 µm filtration. Turbidity measurement showed successful clearance from cell debris in both cases after filtration, while centrifugation alone was not sufficient to clarify the crude lysate. Additionally, SDL proved to be superior with process times of less than 30 minutes for the SDL approach, versus more than 60 minutes for the classical approach (30 min centrifugation + 30 min filtration period).

Figure 4: *Comparison of Turbidity*



Note: Turbidity measured for the different samples shwoing that one-step alluvial filtration is equeal or better than two-step classical clarification, using the Sartoclear Dynamics® Multistation.

Figure 6: *AAV Genomic and Capsid Titer According to Different Clarification Methods*



Note: AAV genomic and capsid titer obtained after cell culture clearance either by centrifugation and subsequent filtration (classical) or after alluvial filtration (SDL), both after chemical cell lysis. Genomic titers revealed product loss for AAV-5 (minor) and AAV-2 (higher degree) caused by filtration in the classical approach. The same effect was observed after alluvial filtration, although to a lower extent and for AAV2 only. This indicates serotype specific loss, potentially associated with AAV aggregation or cell debris-binding tendencies.

Summary | Conclusion

- Production of AAV-2,-5 and -8 in HEK293 suspension culture at bioreactor scale using HEK ViP NB medium and HEK FS feed is easily implemented and results in high titers in the 2-3E14 vg/L range.
- Chemical lysis by e.g., Tergitol enables efficient and scalable cell disruption in suspension-based AAV production.
- Alluvial filtration halves the time required to clear the crude culture lysate.
- Alluvial filtration offers a scalable centrifugation-free clarification of lysed cell harvest which can be superior to classical approaches.
- Superior performance of alluvial filtration approach was especially seen for AAV-2, with less product loss, which can potentially be caused by AAV-2 aggregation and cell debris-binding tendencies.