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 adenovirus-associated virus (AAV) vectors necessitates novel strategies for large-scale manufacturing. While AAV production in suspension allows for easy upscaling, downstream processing of large culture volumes remains challenging. Due to serotype-dependent intracellular localization of AAVs, release of capsids from the producer cell is mandatory and can be accomplished e.g. by multiple freeze-thaw cycles on 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 lysis 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 allowed to grow in a T-150 cm2 flask or in a bioreactor using the SerbioAV™ system. For transient transfection with a two-plasmid system for AAV2, AAV5 and AAV8, the transfection reagent Lipofectamine™ was used for transient transfection with a two-plasmid system for AAV2, AAV5 and AAV8 (Plasmid Factory) with GFP as reporter. For cell lysis, a Tergitol TMN-100x solution 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 (Filtration), or by the addition of diatomaceous earth (Filtration).

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 was monitored by a decrease in viability and viable cell density.

Summary | Conclusion

- Production of AAV-2,-5 and -8 in HEK293 suspension culture at bioreactor scale using HEK VIP NB medium and HEK F5 feed is easily implemented and results in high titers in the 1E14-1E15 vg/mL 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.