Screening of Commercial Production Media and Harvest Depth Filters for AAV Production and Clarification of Transient Transfection Mediated HEK293 Suspension Cell Culture

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Abstract

Adeno-associated virus (AAV) has been widely used as the vector for gene therapy on diseases caused by absent or defective genes. Despite the booming development of AAV's medical applications, current AAV manufacturing is still challenged by low expression level and high cost of goods, which limit its use as a viable therapeutic treatment. One commonly used AAV production approach is the HEK293 suspension cell culture with plasmids transient transfection. Many studies have been performed to improve titer, including host cell screening and engineering, helper plasmid optimization, transfection method optimization, and cell culture process optimization. However, few studies have been published comparing production media and depth filters for harvest clarification from different vendors. In this study, we evaluated the effect of several commercially available HEK293 cell culture media and depth filters on AAV titer expression and clarification respectively. An Ambr15 microbioreactor system was used for AAV cell culture. Cell growth, metabolites, qPCR titer and full capsid percentage were monitored or analyzed. In conclusion, the Expi293™ Expression Medium showed higher expression and full capsid percentage for both AAV8 and AAV9. The Clarisolve and D0SP depth filters showed overall benefit in recovery, throughout and clarification.

Introduction

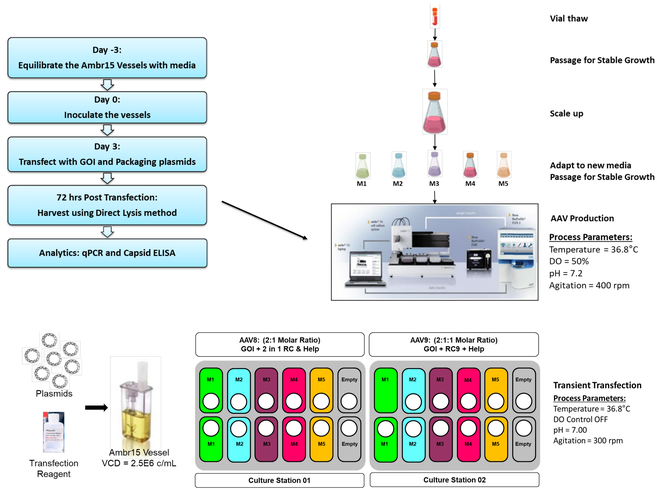
A high-throughout bioreactor-PAT (Process Analytical Technology) platform was used to evaluate the effects of several commercially available HEK293 cell culture media on AAV titer expression. The platform consisted of 24-way Sartorius Stedim Ambr15 microbioreactors integrated with a NOVA Flex2 multi-analyte analyzer. The integrated platform enabled the control and monitor of critical process parameters (CPP) such as pH, dissolved oxygen (DO), temperature and agitation rate. 20 Ambr15 vessels were operated with 5 commercially available medium (12mL working volume), in duplicates. All other process parameters were kept constant. The vessels were monitored automatically daily for cell growth (VCD and Viability) and metabolite concentrations via the NOVA Flex2 analyzer. Once cells grew to the desired density, they were manually transfected in the Ambr15 vessels. The aeration and agitation during transfection were controlled by the Ambr15 system, to limit sheer stress on the complex addition.

Materials

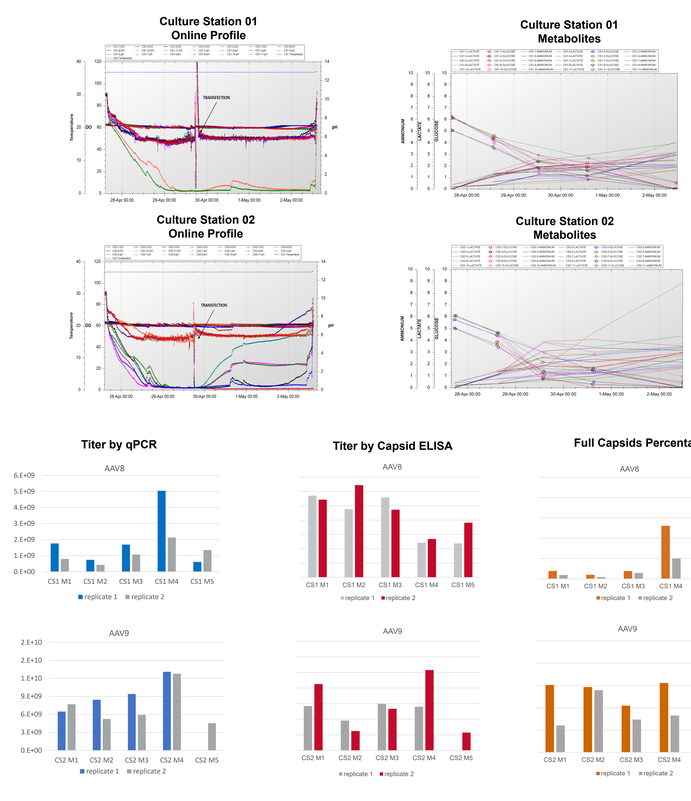
- Expi293F™ Cells
- EX-CELL® CD HEK293 Viral Vector Medium (M1)
- BalanCD HEK293 medium (M2)
- FreeStyle™ F17 Expression Medium (M3)
- Expi293™ Expression Medium (M4)
- BioWhittaker™ 293 Cell Serum-Free Media (M5)
- GlutaMAX™ Supplement
- Gibco™ L-Glutamine (200 mM)
- PEIpro (Polyplus 115-010)

- AAV-GFP expression plasmid
- AAV pDP8 helper+RepCap 8 plasmid
- Agilent pHelper & RepCap 9 plasmids
- AAV lysis buffer – in house
- AAV GFP qPCR assay – in house
- Progen AAV8 & AAV9 Capsid ELISA
- Sartorius Ambr15 Vessels
- Millipore Clarisolve & D0SP depth filters
- Pall Seitz HP Depth filters
- Sartopore 2 0.8/0.45 µm filters

Method



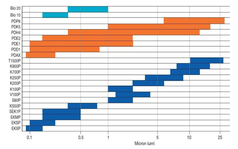
Results



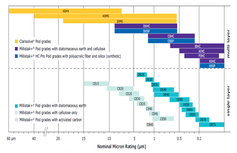
Depth Filtration

Selections of Depth Filters Based on HEK Cell ~13 um in Diameter)

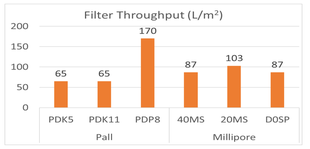
Pall cellulose and diatomaceous earth (DE) based membrane

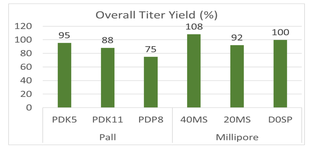


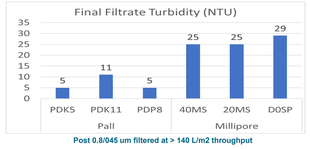
Millipore cellulose, diatomaceous earth (DE) Based, and synthetic membrane



- By P-max method at 20 psid
- By T-max method at 30 NTU







Post 0.8/045 um filtered at > 140 L/m2 throughput

Filter Ranking	1st	2nd	3rd
Titer Recovery	40MS, D0SP	PDK5, 20MS, PDK11	PDP8
Throughput	PDP8	40MS, 20MS, D0SP	PDK5, PDK11
Turbidity	PDK5, PDK11, PDP8	40MS, 20MS, D0SP	

Conclusion

- Ambr15 was used effectively on AAV cell culture conditions screening, with daily temperature and metabolites monitored online.
- qPCR and capsid ELISA assays was used to quantify AAV expression level and full capsid percentage in early stage of process development.
- Using Expi293F™ cell line, Expi293™ Expression Medium produced higher AAV titer and %full capsid than the other commercia media in this study.
- Expression of AAV9 with pHelper plasmid showed higher titer and %full capsid than expression of AAV8 with pDP8 helper plasmid. This is however not observed in adherent cell culture (unpublished data).
- Of all harvest depth filters evaluated in this study, the Clarisolve and D0SP filters showed overall benefits in recovery, throughout and clarification.
- Further process optimization and scale up strategy is required.

References

- Optimization of the HEK293T Suspension Cultivation With a DoE-Approach in Ambr® 15 Cell Culture- Franziska Bollmann, Diana Riethmüller, Erik Johansson, Alexander Tappe, Poster by Sartorius Stedim Data Analytics AB, Sweden. July 2019
- High-titer, serum-free production of adeno-associated virus vectors by polyethyleneimine-mediated plasmid transfection in mammalian suspension cells - Markus Hildinger, Lucia Baldi, Matthieu Stettler, Florian M. Wurm, Biotechnology Letters, 29(11):1713-1721, 17 Jul 2007