Adeno-associated Virus Production in Suspension Cell Culture Using the SciVario® twin Bioprocess Controller

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Abstract

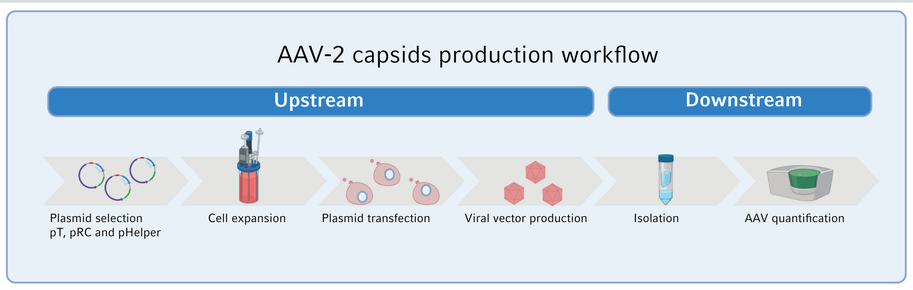
Vaccines are essential tools for resisting pathogens and controlling global pandemics. Various types of virus vaccines, such as live attenuated, inactivated pathogen and subunit vaccines have been widely produced using mammalian cell culture. However, they often rely on attachment cell culture. For higher production capacity, increased yield, and most importantly, ease of production scale-up, it is more desirable to produce vaccines in suspension cell culture platforms. The widely available suspension bioreactors used in antibody production will allow a faster development process of versatile vaccine platforms. Novel technologies such as mRNA vaccines, Adeno virus vector and Adeno-associated virus (AAV) vector-based vaccines have all played a key role in controlling the recent COVID-19 pandemic. Cell culture produced AAV vectors have gained momentum as one of the most effective gene and protein delivery tools in vaccine production as well as gene therapy. The goal of this application note was to demonstrate the feasibility of an efficient and scalable AAV production platform based on suspension cell culture. To that end, a suspension-adapted HEK293 cell line (Expi293F) was used as the host of a Helper-Free AAV system in order to eliminate the requirement for wild-type adenovirus co-infection. To achieve high yield AAV capsid production, the cellular transfection was performed in a BioBLU® 1c Single-Use Bioreactor controlled by the SciVario® twin bioreactor control system. In the course of the experiments, metabolites, cell density and viability, as well as AAV capsid titers were monitored and analyzed. In line with typical industry yields, capsid titers were reaching a robust 10^{12} capsids/mL culture volume.

Equipment

New Brunswick™ S41i CO_2 Incubator Shaker and SciVario twin bioprocess controller

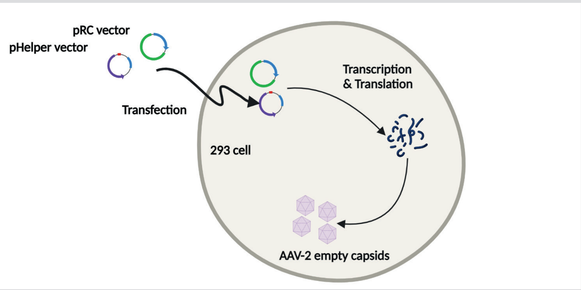
Material and Methods

Typical AAV-2 vector (capsids) production workflow



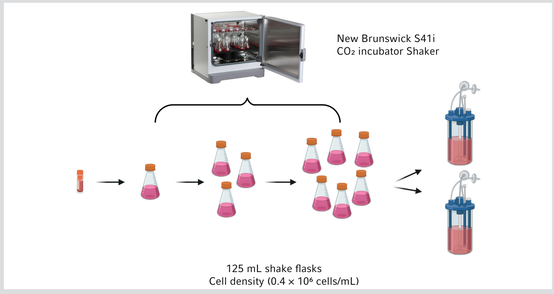
Co-transfection of host cells with plasmids encoding for a gene of interest (pT), AAV structural and packaging proteins (pRC), and helper virus-associated proteins critical for AAV assembly (pHelper) result in the production of viral vectors which are then isolated and quantified for downstream applications.

Production of empty AAV-2 capsids



In order to demonstrate the feasibility of the employed culturing system, suspension-adapted HEK293 (Expi293F) cells were transfected only with pRC and pHelper plasmids to generate empty AAV-2 capsid vectors without a gene of interest.

Workflow AAV suspension culture scale-up



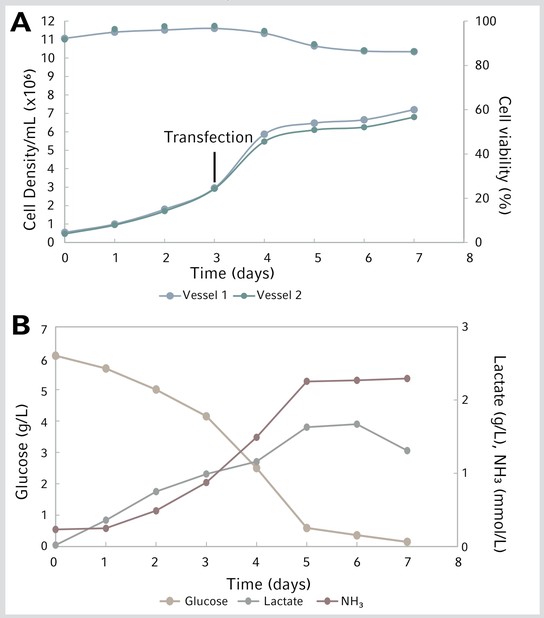
After initial cell expansion in shake flasks using a New Brunswick S41i CO2 incubator Shaker, two BioBLU 1c Bioreactors were inoculated for subsequent plasmid transfection three days later.

Results

Sampling and analytics

Samples from the BioBLU 1c Single-Use Bioreactors were collected daily to determine cell viability, cellular density, and the concentration of the metabolites glucose, ammonia (NH3) and lactate. This was achieved by connecting a sterile 5 mL syringe to the Luer Lock sample port and collecting 3 mL as a viable sample for analysis. 1 mL was used to measure the metabolite levels employing a Cedex® Bio Analyzer (Roche, USA), 1 mL to measure the cell viability and density using a Vi-Cell® XR Viability Analyzer (Beckman Coulter®, USA) and 1 mL to check the pH offline using an Orion Star™ A211 pH meter (ThermoFisher Scientist, USA). 72 hours after transfection AAV-2 capsids were isolated from the Expi293F cell pellet and supernatant before quantifying the capsid titers by ELISA.

Expi293F cell culture in BioBLU 1c Single-Use Bioreactors controlled by the SciVario twin bioreactor control system

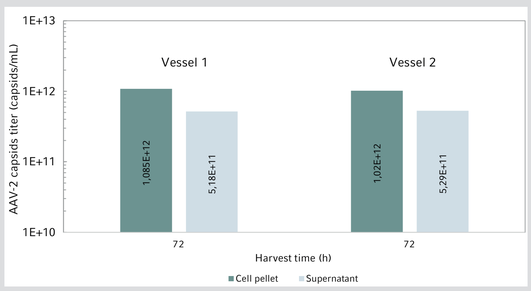


- Rapid cell growth increase in both bioreactors, viable cell density peak of 7 x 10^6 cells/mL **(A)**
- Monitoring the cells' glucose consumption as well as lactate and NH_3 production to maintain lactate and NH_3 levels below 2 g/L and 2.3 mmol/L respectively during the whole run **(B)**

Overview of process parameters for the bioreactor run

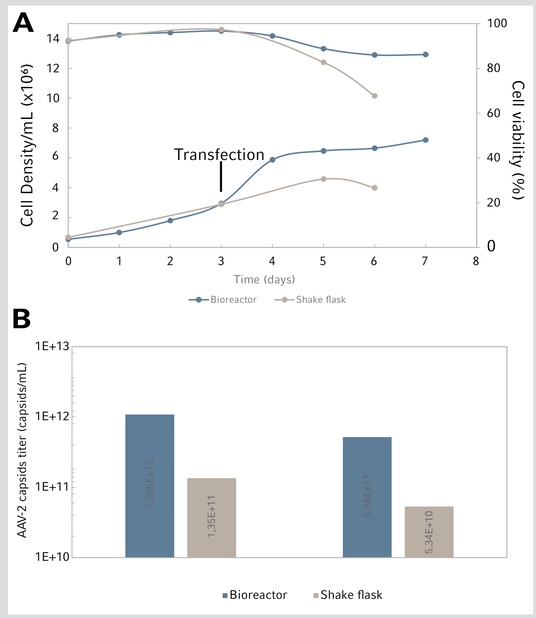
Parameters	Setpoints
Starting volume	800 mL
Ending volume	1 L
Agitation	155 rpm (0.4 m/s tip speed)
Temperature	37 °C
Inoculation density	0.4 x 10^6 cell/mL
Cell culture medium	Expi293™ Expression Medium
DO Setpoint	40% (P=0.1; I=3.6/h)
pH Setpoint	7.0 (deadband = 0.2), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)
Gassing cascade	Set O_2 % at 30 % controller output to 21 % and at 100 % controller output to 100 %. Set flow at 0 % controller output to 0.1 SLPH, and at 100 % controller output to 30 SLPH.

ELISA quantification of AAV-2 capsid titers from cell pellet and supernatant



- Excellent reproducibility due to control by the SciVario twin bioporcess control system as shown by very similar AAV-2 capsid titers from both BioBLU Single-Use Bioreactors
- Higher AAV-2 capsid titers from the cell pellet in comparison to the supernatant

Cell density, viability and AAV-2 capsid titers achieved in BioBLU 1c Single-Use Bioreactors compared to shake flask Expi293F cell cultures



- Higher cell density and viability in the bioreactor process compared to shake flask process after transfection **(A)**
- Much higher capsid titers from the precision-controlled bioreactor system **(B)**

Conclusion

This study underscores a suspension culture-based vaccine production platform as an easier alternative to attachment culture approaches. The suspension culture produced good yield on AAV-2 particles while using the standard stirred-tank suspension bioreactor process that is well established in the field of CHO cell antibody production. The suspension method will allow more straightforward scale-up into larger bioreactors similar to the well-established CHO culture process and offers much desired simplicity, as well as access to a greater variety of production systems over attachment culture-based methods. In this study, the suspension culture process was initiated at the shake flasks stage using a shaking incubator followed by bioreactor suspension culture using the SciVario twin bioreactor control system and BioBLU 1c Single-Use Bioreactors. The efficient and simple setup of the SciVario twin allowed the precise control of the cell culture environment when compared to cell transfection in shake flask, leading to an AAV-2 capsid titer of approximately 10^{12} capsids/mL from the cell pellet compared to the approximately 10^{11} capsids/mL obtained under the same shake flask transfection conditions. Furthermore, the data obtained from both batch culture processes demonstrate the reproducibility of these experiments when using the SciVario twin as a controller. Thus, the Expi293F cell line together with advanced suspension stirred-tank bioreactors and controllers from Eppendorf, such as the SciVario twin, the BioFlo® 320, and the BioFlo 720, offer ideal solutions towards the development of suspension-based vaccine platforms as well as viral vector production for gene therapy R&D.