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Booth：1644

A Robust Upstream Process for AAV8 Production Scaled up from 15 Liters to 1000 Liters

Abstract #1549

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Introduction

In today's medical field, gene therapy is flourishing and garnering significant attention. Adeno-associated viruses (AAVs) have emerged as highly regarded gene therapy vectors due to their low immunogenicity, minimal toxicity, and non-pathogenic nature. However, with the increasing number of AAV gene therapy candidates advancing into clinical and commercial stages, enterprises face a significant challenge: how to successfully scale up the production process of AAV viral vectors from laboratory to industrial scales.

This study aims to explore the successful scaling up of the production process from small-scale bioreactors to the XDR2000 system, achieving a working volume of 1000 liters. Specifically, the research focuses on three key steps: (a) large-scale cultivation of HEK293 suspension cells; (b) mass rapid mixing of DNA-PEIpro complexes; and (c) rapid transfer of large transfection complexes into bioreactors. By optimizing these critical steps, we have successfully achieved efficient production of AAV viral vectors, providing crucial support for further advancements in the field of gene therapy.

Methods

Seed Train of VPC2.0 Cells

Throughout the seed train process, Dynamis medium is employed for cell cultivation. Initially, VPC2.0 cells are thawed in shake flasks and cultured for 12 days. Subsequently, the cell density is expanded to 0.6×10^6 cells/ml and inoculated into two 50 L CELL-WAV Automated cell expansion system, with a total cultivation volume of 50 L. After 3 days, the cell density reaches 6×10^6 cells/ml. The cells are then inoculated into an XDR2000 bioreactor at a density of 0.6×10^6 cells/ml, with a cultivation volume of 450 L. After 2 days, the cells are diluted to 900 L, achieving a density of 1.5×10^6 cells/ml. Within 24 hours, the cells are cultivated to the target density of (3.0±0.3)×10^6 cells/ml, with a cell viability of ≥95%. Throughout the entire cell cultivation process in the XDR2000 bioreactor, measurements are taken for cell density, viability, metabolic parameters, and more. Table 1 presents the operational parameters for a production scale of 1000 L.

Cell Transfection

Transfer three types of plasmid DNA into CELL-WAV Cell Culture Bag containing Dynamis medium. Then, add transfection reagent into the CELL-WAV Cell Culture Bag. After mixing for a certain period, transfer the transfection complex along the vessel wall into the XDR2000 via gravity, with an approximate addition time of 7 minutes.

Virus Harvest

Add lysis buffer for virus harvest 72 hours post-cultivation.

Table 1. Bioreactor Parameters

Parameters	XDR2000
Final Working Volume	1000 L
Cultivation Temperature	37.0℃
pH	7.0±0.25
pH Control	CO2 Introduction via 1 mm Perforated Gas Distributor
DO	40%
DO Control	Air and Oxygen Introduction via Cascade of 1 mm and 20 μm Gas Distributors
Target Transfection Density	(3.0±0.3)×10^6 cells/ml, Cell Viability ≥95%

Results and Discussion

In the process of producing AAV8 with a final working volume of 1000 L in the XDR2000, various cultivation monitoring indicators were observed. These include the growth curve of VPC2.0 cells depicted in Figure 1a, where cell density and cell viability were tracked. Additionally, Figures 1b, 1c, and 1d illustrate the monitoring of glucose concentration, lactic acid concentration, and ammonium ion concentration, respectively, throughout the production process within the XDR2000. These monitoring efforts ensure the precise control and optimization of critical parameters for maintaining the stability and consistency of the production process.

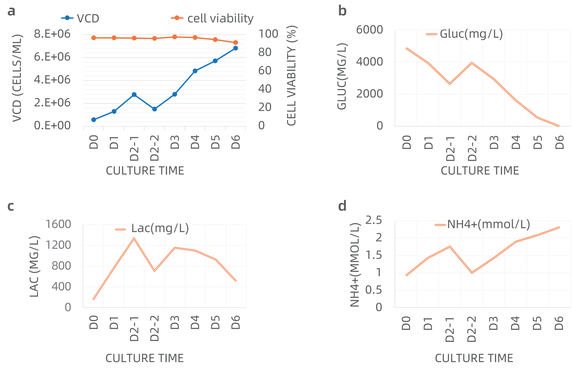

Figure 1 Monitoring indicators throughout the production process. a. Growth curve of VPC2.0 cells in the XDR2000. b. Glucose concentration curve c. Lactic acid concentration curve. d. NH4+ concentration curve.

In XDR2000, AAV8 was produced with a final working volume of 1000 L. The upstream crude product genome titer, drug substance solid rate after purification, HCD residue, and HCP residue were compared with those of XDR200. Figure 2a illustrates the comparison of upstream crude product genome titers, while Figure 2b compares the solid rates of drug substance after purification. Additionally, Figure 2c presents the comparison of HCD residues in the drug substance, and Figure 2d shows the comparison of HCP residues in the drug substance.

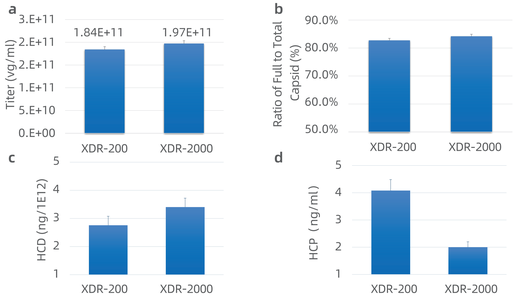

Figure 2 Parameters in the production process. a. Comparison of upstream crude product genome Titers. b. Comparison of drug substance solid rates after purification. c. Comparison of drug substance HCD residues. d. Comparison of drug substance HCP Residues.

Conclusion

The AAV8 titers obtained in the XDR2000 bioreactor were comparable to those obtained at scale using XDR200, demonstrating successful scale-up of AAV production to commercial manufacturing scale. This achievement meets the demand for future commercial production of AAV products.

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About OBiO

OBiO Technology leads the way in gene and cell therapy as a pioneering Contract Research Organization (CRO) and Contract Development and Manufacturing Organization (CDMO), dedicated to providing comprehensive solutions. Our state-of-the-art 830,000-square-meter Facility for Global Supply epitomizes our commitment to meeting global demand. We specialize in vectorology studies, functional genomics, and process and analytics development, ensuring Investigational New Drug (IND) readiness and supporting all phases of clinical and commercial manufacturing. Guided by our mission to "enable gene and cell therapy for better lives ," we prioritize delivering top-tier services worldwide. From laboratory to clinic, we continuously advance your products, positively impacting populations worldwide. Our unwavering commitment ensures the delivery of high-quality CDMO services across the preclinical, IND, clinical, and commercial stages. Our expertise spans plasmids, mRNA, AAV, LVV, Ad viral vectors, cell therapy, CAR-T, NK, Treg, as well as cutting-edge technologies like inducible viral vector packaging, ultralow endotoxin processes, and AAVneO for tissue-specific AAV variant

About OBiO TECH USA

OBiO TECH, INC is headquartered in Pleasanton, California, and was established in February 2023. This site accommodates both our Research & Development (R&D) and Business Development (BD) teams. Our BD team is led by a seasoned senior BD professional with decades of experience in the therapeutic/pharmaceutical industry. Meanwhile, our R&D team is helmed by experts in the gene and cell therapy field, boasting extensive academic and industry backgrounds. This location serves as a strategic hub for expanding our global business operations to meet the diverse needs of our customers and foster international research collaborations in the gene and cell therapy domain.