Scalable Bioprocessing Strategies for Intensified Viral Vector Production in HEK293 Cell Culture

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INTRODUCTION

Scalability in bioprocessing is critical to ensure that bioprocesses developed at small scales translate to larger scales without compromising product quality, yield, or consistency in cell growth kinetics. Maintaining key culture conditions, such as nutrient supply, mass transfer, and mixing, is essential for achieving consistent cell growth, metabolic behavior, and viral vector production. This work explores how intensified cell perfusion systems can work hand in hand with cell culture media development to address these challenges, with a focus on scale-up strategies that ensure reproducible cell growth and viral vector production across different scales in HEK293 cell cultures.

The specialized media we developed for the HEK293 perfusion process enabled us to optimize nutrient balance and waste management efficiently. With a cell-specific perfusion rate (CSPR) as low as 25 pL/cell/mL, we developed a perfusion ramping strategy that achieved 1.1 x 10^8 cells/mL with over 90% viability, without experiencing a depletion of key nutrients or a build up of harmful byproducts. In addition, using power input over volume (P/V) as the key factor to ensure consistent cell growth across scales and a specific transfection protocol, we successfully achieved intensified viral vector production by transiently transfecting cells at 2 x 10^7 cells/mL across various perfusion processes and bioreactor scales. Automated control systems, driven by real-time sensor feedback, were employed to optimize key process parameters such as gassing, nutrient delivery, metabolite removal, and shear rate across cell retention devices. By maintaining precise control of bioprocess parameters, we achieved consistent HEK293 cell metabolic profiles across different cell densities and scales, demonstrating the robustness and versatility of our bioprocess. This approach successfully reproduced HEK293 cells metabolism and viral vector production from 50 mL to 5 L scales.

This work provides a scalable bioprocess that ensures reproducibility in cell growth and viral vector production, serving as a blueprint for improving viral vector manufacturing workflows.

MATERIALS AND METHODS

Cell stock and growth medium: Suspension HEK293 cells and in-house developed media (FUJIFILM Irvine Scientific)

Culture vessels and culture conditions:

- **125 mL Corning shake flasks:** 37°C, 5.0% CO_2, 130 RPM, 80% humidity
- **50 mL Corning mini bioreactor spin tubes:** 37°C, 5.0% CO_2, 250 RPM, 80% humidity
- **Ambr15 micro bioreactor vessels or Dasgip bioBLU 3c single-use vessels:** 37°C, 5.0% CO_2, 40% DO set-point, unless indicated otherwise, no pH control was used

Seeding density for growth phase: 0.5 x 10^6 cells/mL with > 95% viability, or as indicated

Cell counting method: 200 µL sample for viable cell density (VCD) and viability; Vi-Cell BLU (Beckman Coulter)

Cell density at transfection: 2 x 10^7 cells/mL

Passage number used: P3–P20

Transfection reagent: Polyethylenimine (PEIpro, PolyPlus)

DNA: Triple transfection with AAV2 (GOI/gene of interest: ZsGreen, RC: Rep-Cap, and helper plasmids) with 1:1:1 molar ratio with a total DNA concentration of 0.4 µg:1 M cells

DNA:PEI: Ratio at 1:2

QIAcuity Digital PCR system (Qiagen): Using ITR primers

RESULTS

Maximum VCD with Perfusion in Bioreactors

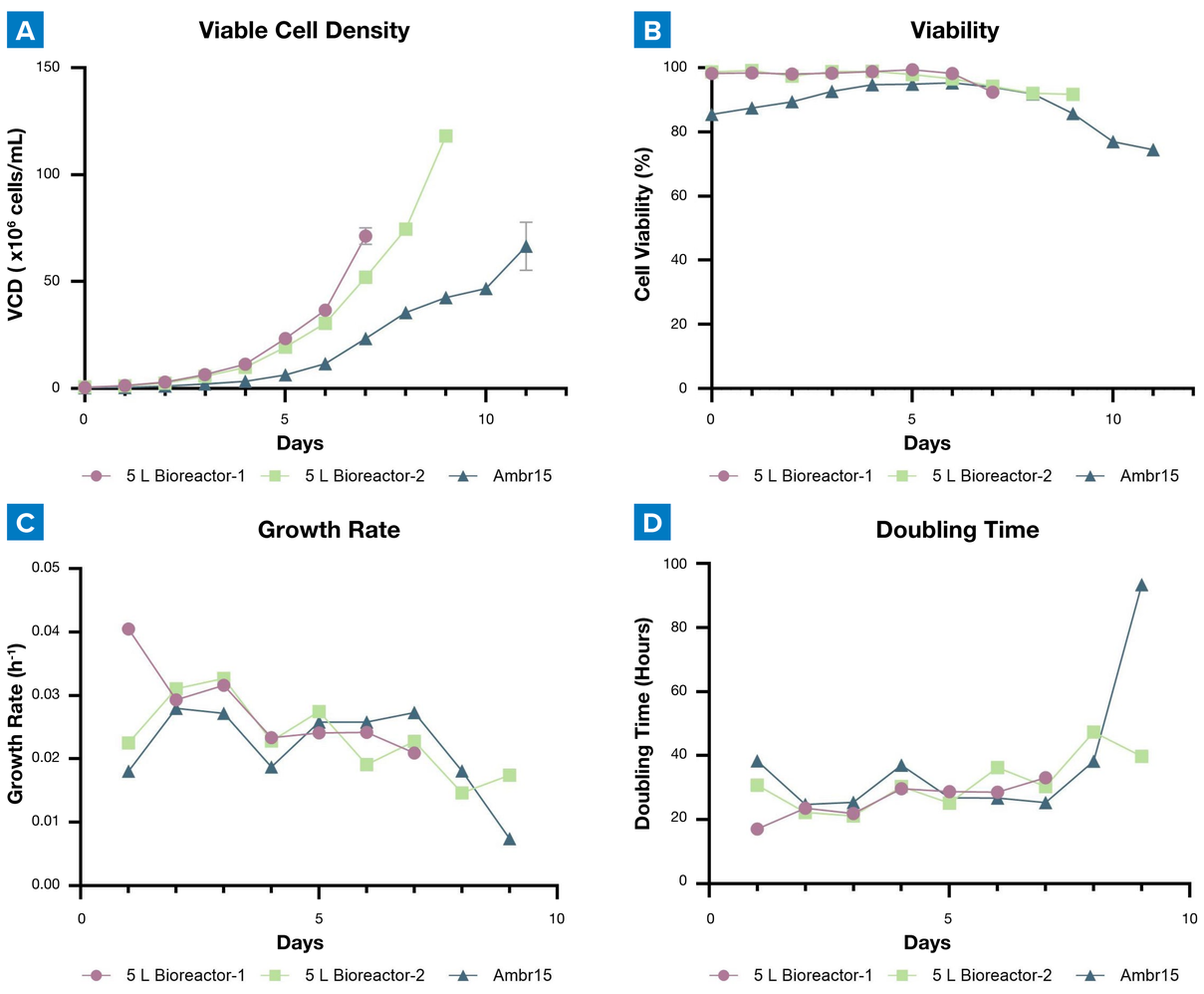

Figure 1. Maximum VCD perfusion study in Ambr15 and Dasgip BioBLU 3c Bioreactor. Suspension HEK293 cells were seeded into Ambr15 mini bioreactors with a working volume of 13 mL and into Dasgip BioBLU 3c bioreactor with a working volume of 3.3 L. A perfusion-mimic strategy was used to maintain 1 vvd in the Ambr15 bioreactors; spent media was removed using centrifugation and fresh media was replenished in the bioreactor by manual addition. A constant CSPR of 25 pL/cell/day was maintained in Dasgip bioreactors throughout the entire experiment. Alternating tangential flow (ATF) using ATF2 system (Repligen) with 0.2 µm hollow fiber was used to perfuse in the Dasgip bioreactor. **(A)** VCD, **(B)** Cell viability, **(C)** Cell growth rate (h^{-1}), and **(D)** Doubling time (hours) during the exponential growth.

Lactate and Ammonium Profiles Using Suboptimal Formula

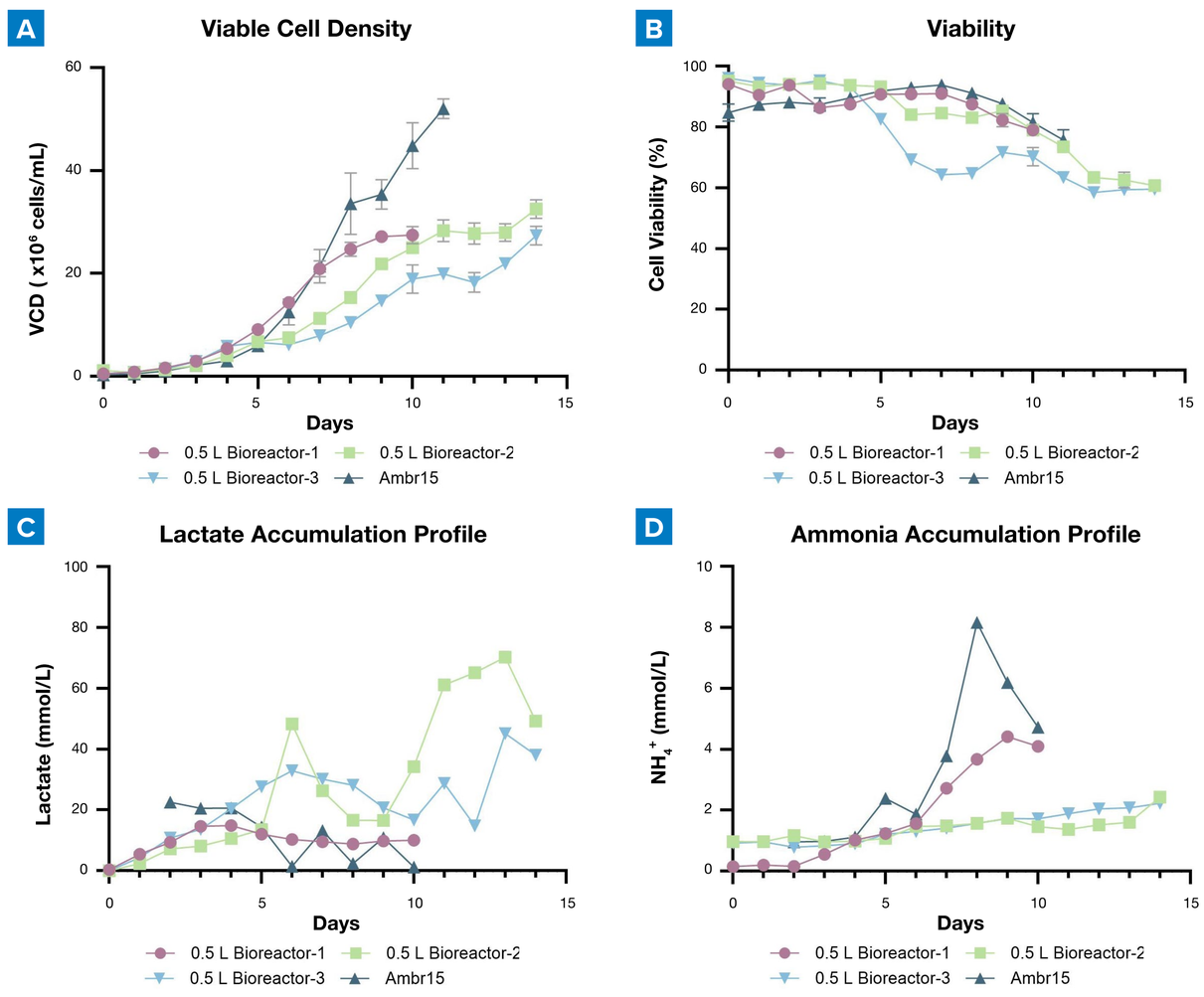

Figure 2. Lactate and ammonia profile during cell culture. Suspension HEK293 cells were seeded into Ambr15 bioreactors with a working volume of 13 mL and into a custom 0.5 L bioreactor with a working volume of 250 mL. 0.5 L Bioreactor-1 and the Ambr15 bioreactor were cultured without pH control, while 0.5 L Bioreactor-2 and -3 were cultured with a pH set point of 7.0 ± 0.2. A perfusion-mimic strategy was used to maintain 1 vvd in the Ambr15 bioreactors; spent media was removed using centrifugation and fresh media was replenished in the bioreactor by manual addition. Alternating tangential flow using 0.2 µm hollow fiber (Repligen) equipped with a custom flow path was used to perfuse the 0.5 L benchtop bioreactor. **(A)** VCD, **(B)** Cell viability, **(C)** Lactate accumulation, and **(D)** Ammonia accumulation.

Formula and Process Optimization for Enhanced Culture Performance

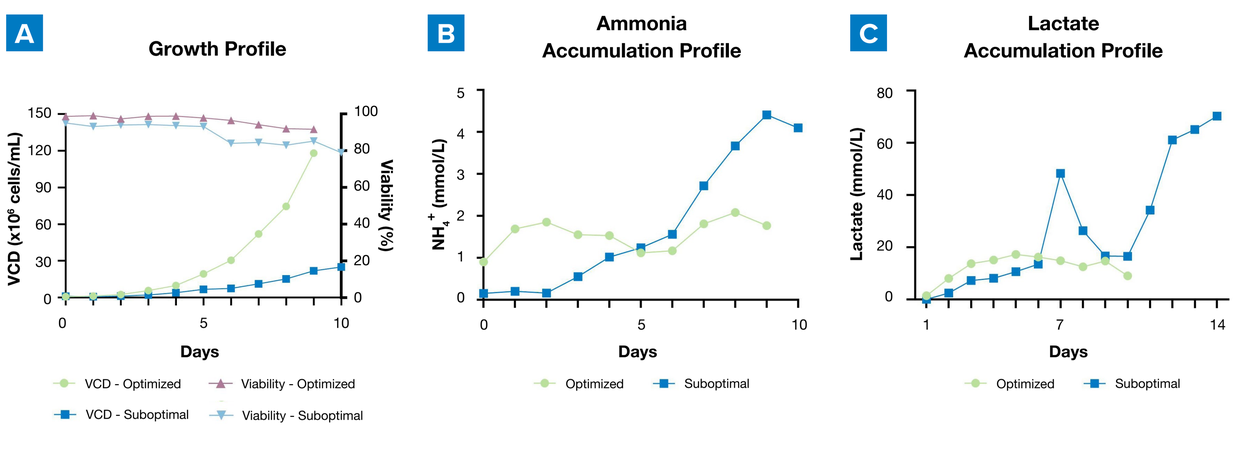

Figure 3. Enhanced performance through formulation and bioprocess optimization. Suspension HEK293 cells were seeded into 2 benchtop bioreactors under either pH-controlled conditions (7.0 ± 0.2) with a non-optimized formula or no pH control with an optimized formula. The pH-controlled bioreactor operated at a fixed perfusion rate of 1 vvd, while the no pH control bioreactor maintained a constant CSPR of 25 pL/cell/day. **(A)** Growth profile (VCD and viability), **(B)** Ammonia accumulation, and **(C)** Lactate accumulation.

Media Optimization Yield Improved Metabolite Profiles

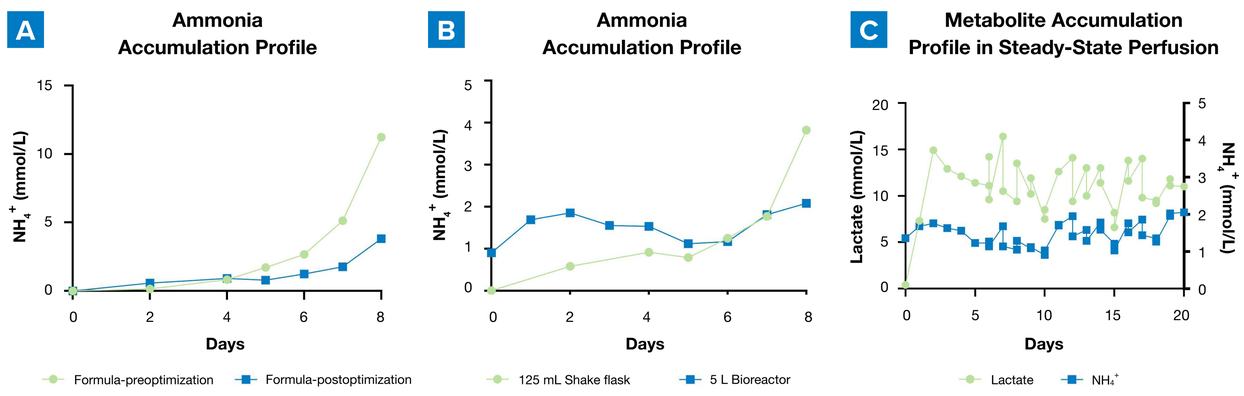

Figure 4. Impact of cell culture media optimization. **(A)** Ammonia accumulation was compared between 2 media formula (preoptimization and post). **(B)** Ammonia accumulation was assessed in low cell density shake flask cultures and high cell density bioreactor cultures (> 10x the cell density of the shake flask) using the optimized formula. The shake flask cultures were grown in batch mode, while the bioreactor was equipped with ATF at a constant CSPR of 25 pL/cell/day. **(C)** Lactate and ammonium concentrations were monitored in a steady-state perfusion bioreactor at 4 x 10^7 cells/mL using ATF at a constant CSPR of 25 pL/cell/day with the optimized formula.

Reproducible AAV2 Titer During Scale-up

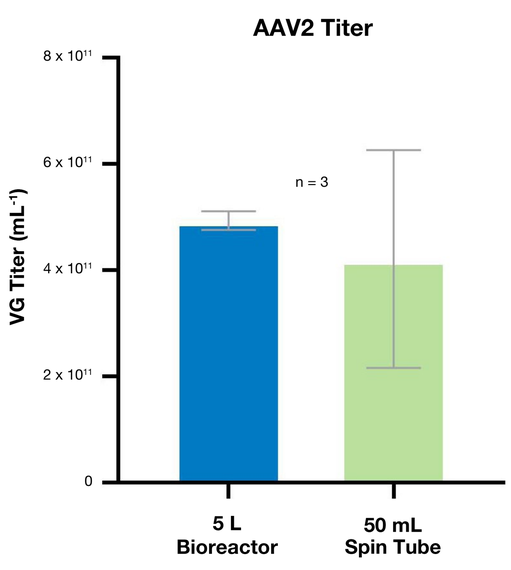

Figure 5. Production of AAV in high cell density transfection in Dasgip BioBLU 3c Bioreactor vs. 50 mL mini bioreactor spin tube. Suspension HEK293 cells were seeded into a Dasgip BioBLU 3c Bioreactor with a 1.3 L working volume (pre-transfection) and maintained at a CSPR of 25 pL/cell/day throughout the experiment. Cells were transfected at approximately 2 x 10^7 cells/mL VCD using AAV2 plasmids via a proprietary method. In parallel, suspension HEK293 cells were seeded at 2 x 10^7 cells/mL (> 95% viability) into 50 mL mini bioreactor spin tubes with a 10 mL working volume. Transfection was performed using the same material and method, with a perfusion-mimic setup achieved through manual daily media exchange (1 vvd).

SUMMARY

Optimizing both bioprocess parameters and media formula is crucial for improving cell culture performance and production. Our studies demonstrate that eliminating pH control combined with media optimization significantly enhances cell growth, viability, and metabolite control.

Key findings include:

Bioprocess and media optimization: Avoiding pH control and optimizing formula in perfusion condition strategies minimized lactate buildup and led to a threefold reduction in NH_4^+ accumulation, supporting cell densities up to 1.18 x 10^8 cells/mL with a cell-specific perfusion rate as low as 25 pL/cell/day.

Steady-state perfusion: Cells maintained a stable metabolic profile in steady-state perfusion for 14 days, with consistent NH_4^+ and lactate levels at 4 x 10^7 cells/mL.

Scalability of AAV production: High cell density transfection in both a Dasgip BioBLU 3c Bioreactor (1.3 L) and 50 mL mini bioreactor spin tubes showed comparable AAV titers, demonstrating process scalability.

By integrating optimized bioprocess strategies with enhanced media formula, we achieved improved cell culture efficiency and robust AAV production, highlighting the potential for large-scale manufacturing applications.