Demonstration of Scalable AAV Vector Production in Kuhner SB10-X Orbital Shaken Bioreactor Using a Novel Human Amniotic Epithelial-Derived HAT Cell Line

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Introduction

Adeno-associated virus (AAV) has emerged as a leading platform for gene therapy; however, achieving high-yield and high-quality production at scale remains a major challenge. To address this, we developed a novel Human Amniotic epithelial cell line for gene and cell Therapy (HAT cells) specifically tailored for AAV vector manufacturing. HAT cells were established from placental amnions obtained from multiple healthy donors undergoing scheduled cesarean sections, with informed consent and in compliance with applicable regulatory frameworks. Each donor sample was processed independently, resulting in the generation of over 500 candidate lines that capture broad biological diversity. From this panel, a lead A2C1 clone was selected based on rapid proliferation and robust AAV production performance. Here, we demonstrated the scalability of HAT A2C1 cells using Kuhner SB10-X orbital shaken bioreactor across shake flask, 3 L, and 10 L bioreactor systems.

Establishment and Development of HAT Cells

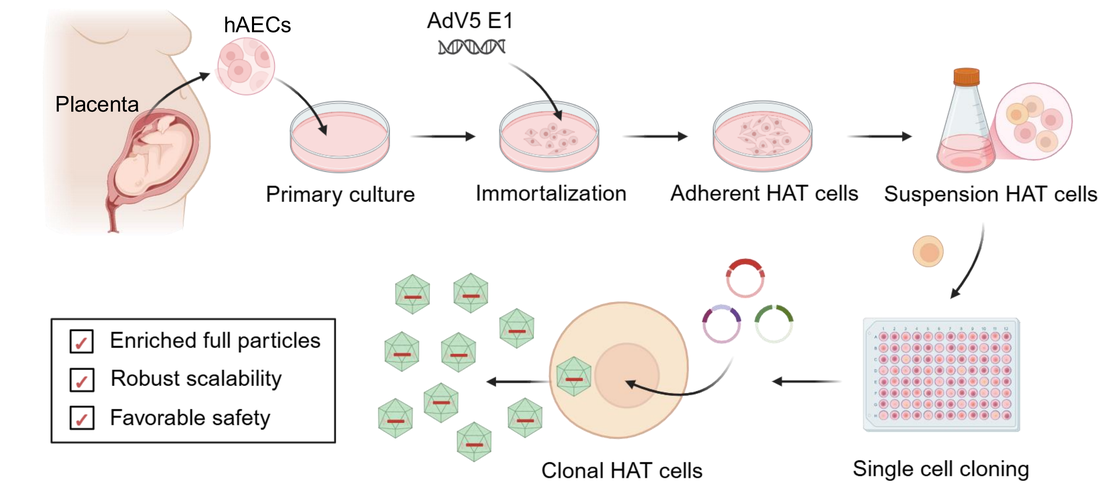

Figure 1. Schematic overview of the establishment and development process of the HAT cells.

Scalable Culture of HAT Cells in SB10-X

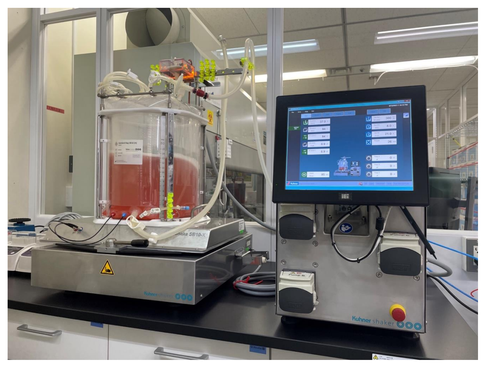

Figure 3. (A) Representative image of HAT A2C1 cell cultures at scale. (B) Expansion culture of HAT A2C1 cells from shake flask to a 4.4 L working volume in the SB10-X bioreactor, showing stable cell growth and cell viability over a 24-day culture period.

Identity and Safety of HAT Cells

Assay	Result
Karyology Analysis	Human Origin
CO1 Barcode Identity	Homology with Human
Sterility Testing (EP, USP, and JP)	Pass
Mycoplasma Detection	Non-detected
Bovine/Porcine Viruses	Non-detected
Specific Human Pathogens	Non-detected
Adventitious Viruses	Non-detected
Transmission Electron Microscopy for Extraneous Agents	Non-detected

Table 1. Summary of identity and safety characterization of HAT A2C1 cells performed by an independent third-party laboratory.

Scalability of HAT Cells at 3 L Scale

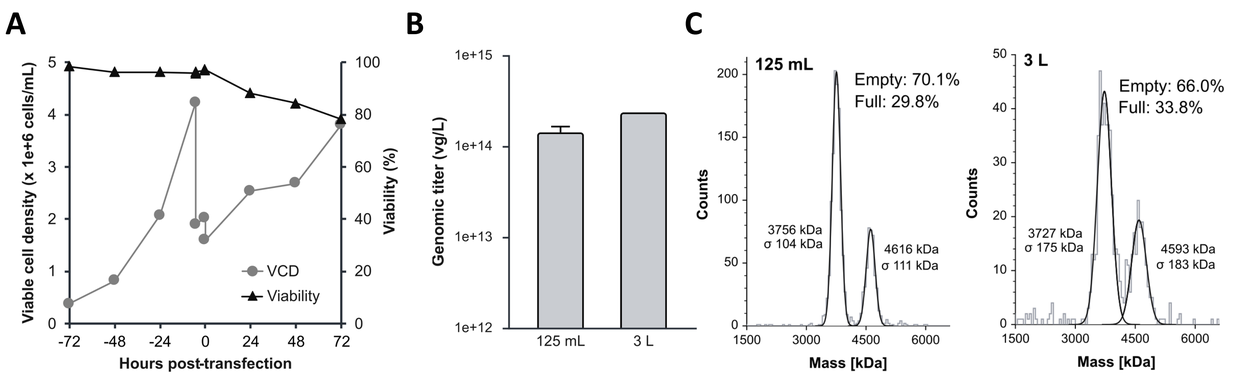

Figure 4. (A) HAT A2C1 cells maintained robust proliferation and high viability in a 3 L bioreactor culture. AAV8 vectors were produced using triple plasmid transfection (GOI: ZsGreen1) with FectoVIR-AAV. (B) Comparable AAV8 genomic titers were observed between 3 L bioreactor and shake flask cultures. (C) AAV vector quality was maintained across scales.

Performance of HAT Cells

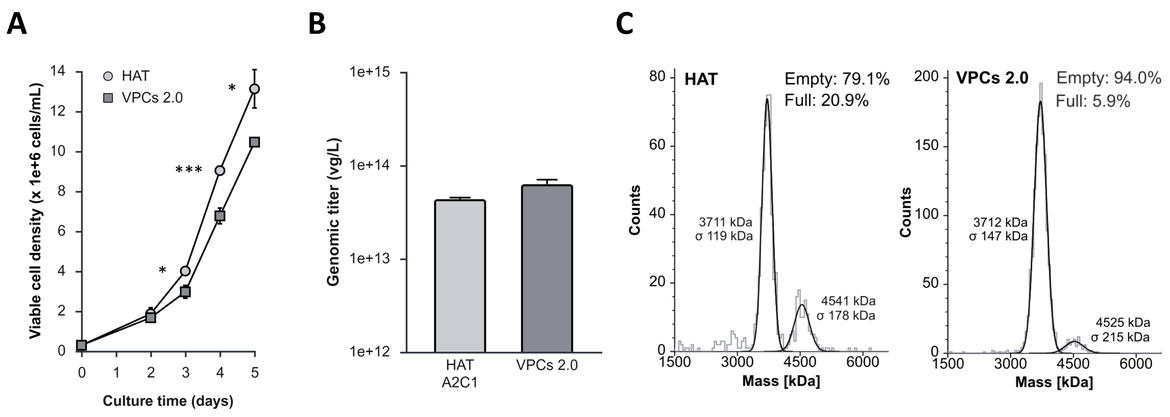

Figure 2. Baseline performance characterization of HAT A2C1 cells in serum-free suspension culture in 125 mL shake flasks. (A) Cell proliferation profiles compared with a representative HEK293-derived production cell line. Both cells were adapted to HE400AZ medium (Gmep). (B) AAV2 vector production using triple plasmid transfection (GOI: ZsGreen1) with FectoVIR-AAV (Polyplus). Genomic titers were measured by ddPCR (Bio-Rad). (C) Assessment of the AAV product quality based on empty-to-full particle ratios by mass photometry (Refeyn). *p<0.05, ***p<0.005.

Performance Validation of HAT Cells at 10 L Scale

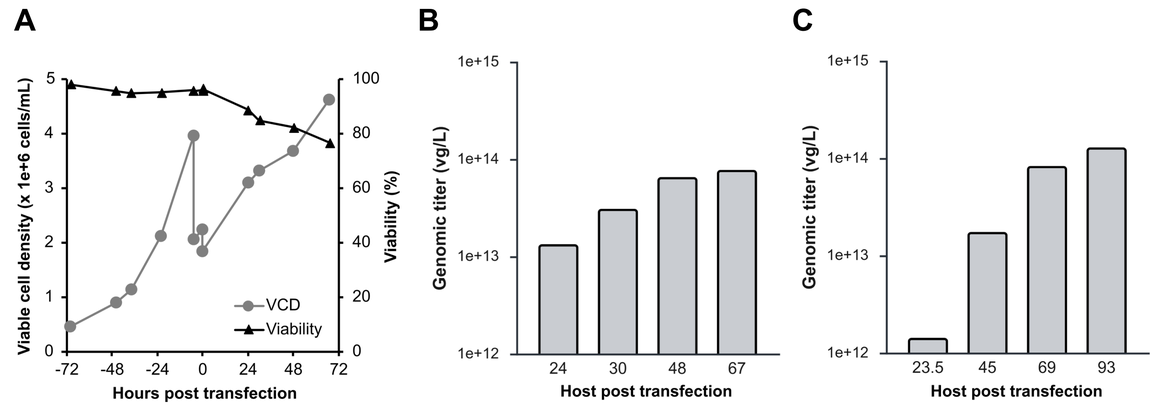

Figure 5. (A) HAT A2C1 cells maintained robust proliferation and high viability in a 10 L bioreactor culture. AAV vectors were produced using triple plasmid transfection (GOI: ZsGreen1) with FectoVIR-AAV. (B) AAV2 vector production resulted in high genomic titer during the production period. (C) AAV5 vector production showed sustained increases in genomic titer over the culture period.

Summary

HAT A2C1 cells enable efficient AAV vector production with an increased proportion of full particles. In addition, HAT A2C1 cells readily adapt to bioreactor conditions without additional optimization, maintaining robust growth and AAV production performance. Using the Kuhner SB10-X bioreactor, scalable AAV production was demonstrated, with comparable AAV titers and empty-to-full particle ratios observed between 125 mL shake flask and 3 L cultures, and consistent cell growth and AAV productivity preserved upon further scale-up to 10 L. Together, these results highlight the potential of HAT cells as host cells for AAV vector manufacturing.

Reference

Hirai Y, Chang Y-H, Yamamoto A, Asahina R, Moromizato R, Kinoshita M, Aizawa K, Miyai M, Kubota M, Horiuchi T, Nakamura K,
Establishment of a novel human amniotic epithelial-derived cell line, HAT, for high-yield AAV vector production,
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This research was supported by AMED under Grant Numbers JP18ae0201001 and JP24se0123004.

This research was conducted under “Ethical Guideline for Life Science and Medical Research Involving Human Subjects” (MEXT, MHLW, and METI Notification No. 1 of 2021, the Government of Japan).