



A VERSATILE AND SCALABLE MEDIA PLATFORM DESIGNED FOR CELL BASED APPLICATIONS USING HUMAN EMBRYONIC KIDNEY 293 CELLS

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Introduction

Human embryonic kidney cells (HEK293) are a well-established cell line for transient protein expression due to ease of culture, scalability, and transfection, and has been garnering greater interest for its use in viral vector production for various applications, including gene therapy. However, there are few commercially available media designed specifically for both transient protein expression and viral vector production. Presented here is a chemically defined, animal component free system known as BalanCD® HEK293 comprising of growth medium, feed medium, and anti-clumping supplement developed for HEK293 cell based applications. Several suspension HEK293 cell lines as well as multiple applications including lentivirus, adeno-associated virus (AAV), and protein production were compared with other established media, demonstrating the advantages of BalanCD HEK293. As the BalanCD HEK293 system allows for one medium to be used throughout the entire process from cell maintenance and expansion to transfection and production, users can seamlessly transition from stage to stage and from benchtop to large-scale studies without the need for switching medium. Furthermore, based on the application, the feed medium and anti-clumping supplement can be added for enhancement of cell growth and protein production and to minimize cell aggregation, respectively. This versatile and scalable media system simultaneously supports a range of applications while maintaining optimal production yields.

Methods

AAV and Lentivirus Production

- Cell stock: HEK293 (AAV) and HEK293T (lentivirus)
- Culture vessel: benchtop bioreactor with 2 L volume
- Seeding density: 3×10^5 HEK293 and 1.5×10^5 HEK293T cells/mL
- Transfection agent: Polyethylenimine (Polyplus, Catalog# 115-010)
- 3 plasmids for AAV and 4 plasmids for lentivirus
- DNA:PEI ratio of 1:1.5
- Total amount of 2 μ g (AAV) and 2.5 μ g (lentivirus) DNA per 10^6 cells

Protein Expression

- Cell stock: HEK293-6E (National Research Council Canada)
- Culture vessel: 125 mL baffled shake flask (Corning, Catalog# 431405) with 30 mL working volume
- Seeding density: 3×10^5 cells/mL
- Transfection agent: Polyethylenimine (Polysciences, Inc., Catalog# 23966-1)
- Hc, Lc of biosimilar antibody, AKT, and GFP at 40%, 40%, 15%, and 5%, respectively (National Research Council Canada)
- DNA:PEI ratio of 1:3
- Total amount of 0.5 μ g DNA per 10^6 cells

Medium Selection

Ten commercially available media were screened and Supplier A was chosen for further evaluation because it was the leading medium compared to other media

Results

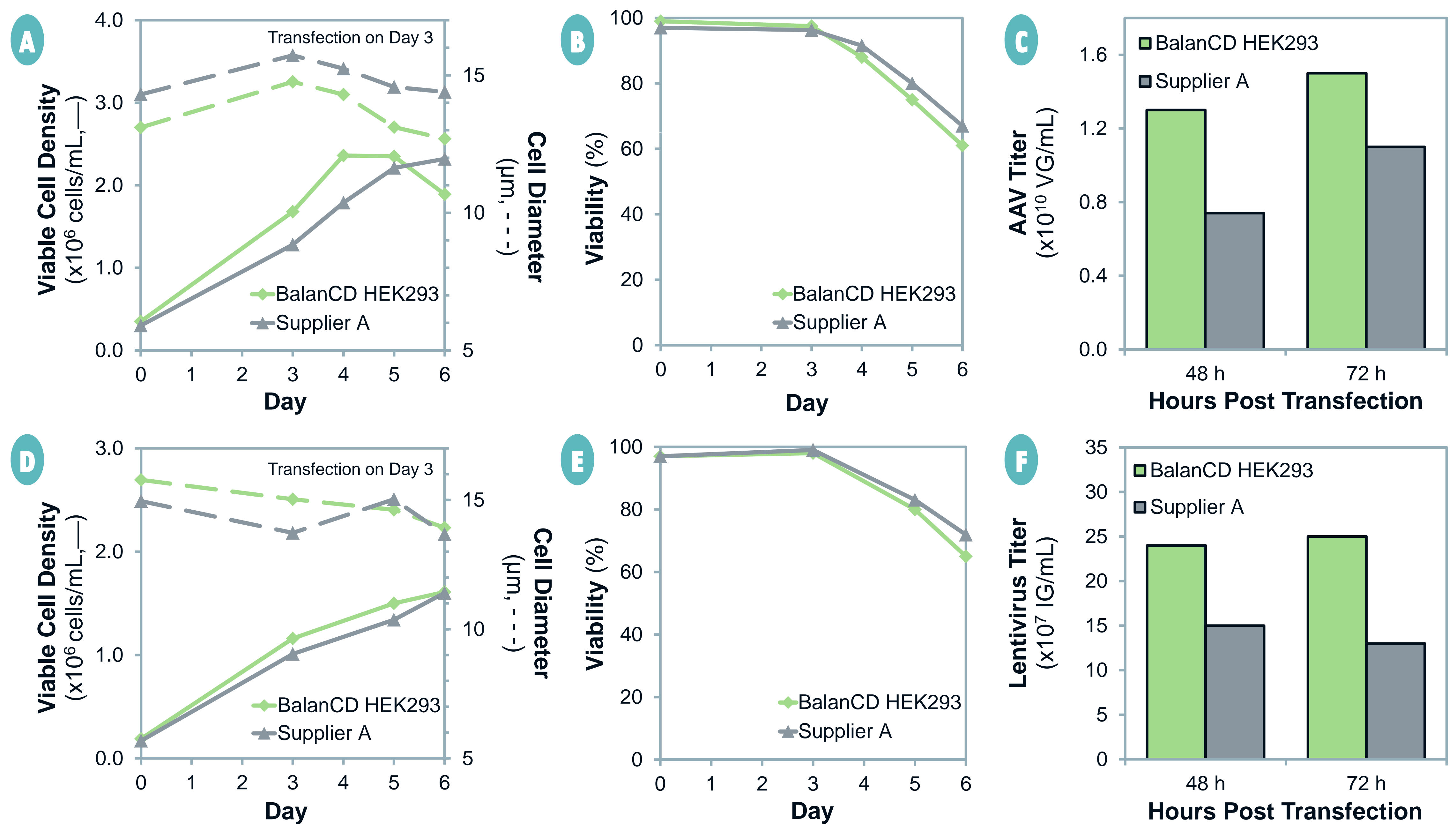


FIGURE 1. Improved AAV and lentiviral vector production in HEK293 cells cultured in BalanCD HEK293 medium. HEK293 (AAV) and HEK293T (lentivirus) cells were thawed directly into each medium and passaged every 3 to 4 days for three passages before going into a 2 L benchtop bioreactor production run. Cells were seeded and cultured for 3 days before being transfected. Viable cell density and cell diameter (A, AAV; D, lentivirus) and percent viability (B, AAV; E, lentivirus) were measured. AAV titer (C) and lentiviral titer (F) were measured 48 and 72 hours post transfection. For AAV, the culture supernatants were collected and treated with DNase before DNA extraction and the vector genome copy number was quantified by qPCR. For lentivirus titer, the culture supernatants were collected and treated with DNase and serially diluted before inoculation on HCT116 cell monolayers. One day later, the cells were trypsinized and lysed for DNA extraction. The integrated vector genomes were quantified by qPCR.

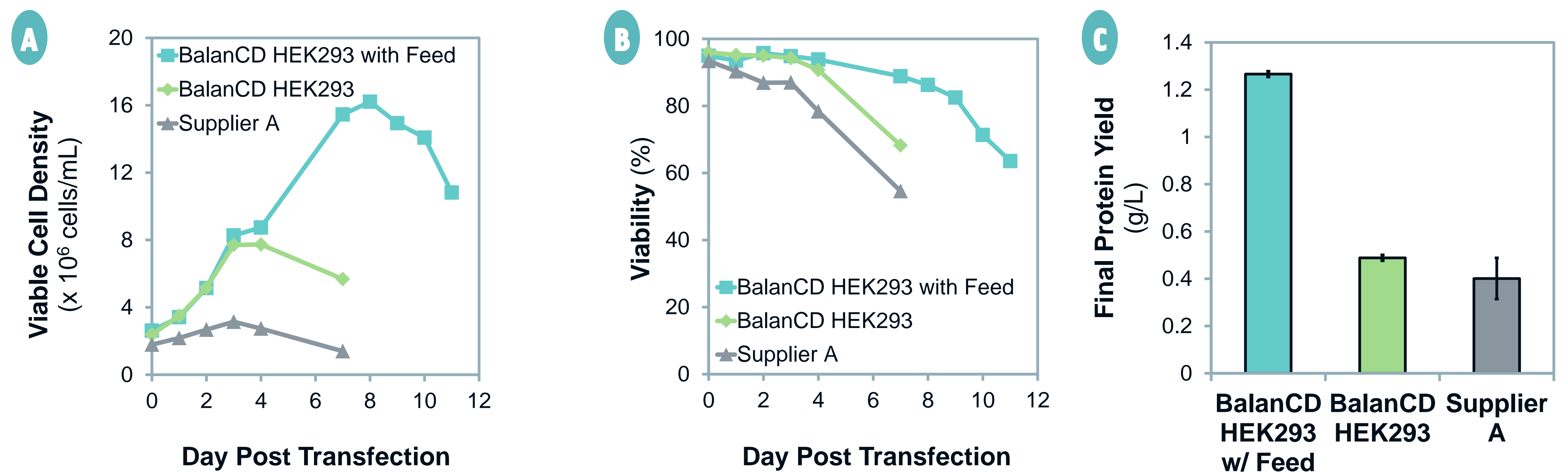


FIGURE 2. Enhanced cell growth and transient protein expression using the BalanCD HEK293 system with HEK293-6E cells. HEK293-6E cells were seeded and cultured for 3 days before being transfected at a cell density of 1.5 to 2.0×10^6 cells/mL in baffled 125 mL shake flasks. Viable cell density (A) and percent viability (B) were measured. On day 1 post-transfection, transfection efficiency was measured using flow cytometry and was approximately 40% GFP positive for all conditions (data not shown). BalanCD HEK293 was supplemented with a 5% v/v addition of BalanCD HEK293 Feed on days 1-4 post-transfection. When conditions reached a viability below 70%, they were terminated and harvested. Final protein yield (C) was measured on day 7 for the batch conditions (BalanCD HEK293 and Supplier A) and on day 11 for the fed batch condition (BalanCD HEK293 Feed).

Summary

- Superior AAV and lentiviral vector production were observed using BalanCD HEK293 medium when compared to the leading commercially available medium resulting in a 40% increase for AAV and 60% increase for lentiviral production.
- BalanCD HEK293 in combination with the BalanCD HEK293 Feed can enhance cell growth and transient protein production resulting in > 1 g/L yield.
- The BalanCD HEK293 system offers a versatile and scalable platform that can be used for a range of applications.

Acknowledgments

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