Development of a Feed Medium to Improve AAV Production in HEK293 Fed-Batch Culture

Omid Taghavian,* Hamid Soleymani, Tannaz Goodarzi, Shahram Shahabi, and Chandana Sharma
Department of Research and Development, FUJIFILM Irvine Scientific, 1830 E. Warner Ave., Santa Ana, CA 92705, USA
*omid.taghavian@fujifilm.com

BACKGROUND AND NOVELTY

Recombinant adeno-associated virus (AAV) is a vector for gene therapy that elicits robust and long-term gene expression, minimal immunogenicity, little to no toxicity, and availability of various serotypes with different cell tropism. Among several approved rAAV-based medicine and numerous ongoing clinical trials, rAAV is the leading choice vector for gene delivery platforms.

The industry standard for AAV production is using HEK293 cells and triple plasmid transfection. Current production yields cannot satisfy the high demands of commercial vector manufacturing. Interestingly, a majority of the produced recombinant capsids are genome-free, which is not useful for gene therapy, but also complicates downstream processing and enhances the risk of immune response induction.

These factors are part of the reason for the high cost of gene therapy products. Thereby, rAAV-based therapeutics can easily cost several thousand to millions of dollars for a course of treatment.

Here, we introduce a feed medium that upon applying to HEK293 cell culture post-transfection, boosts the AAV production, improves the packaging efficiency, and keeps the cell health indices.

MATERIALS AND METHODS

- **Cell stock:** Expi293 (HEK293F, Thermo Fisher Scientific)
- **Growth medium:** BalanCD HEK293 (FUJIFILM Irvine Scientific)
- **Culture vessel:** 24-mL deepwell plate/125 mL and Corning shake flask/10 L XDR bioreactor
- **Seeding density for growth phase:** $3x10^6$ cells/mL
- **Seeding density for transfection phase:** $1x10^6$ cells/mL, 24 h prior to transfection
- **Passage number used:** 3-25
- **Transfection reagent:** Polyethylenimine (PEIpro, PolyPlus)
- **DNA:** Triple AAV2 plasmids (GOI: Gene of interest, RC: Rep-Cap, and Helper plasmids) with 1:1:1 molar ratio. Total amount of 1.5 µg/mL, DNA: PEI ratio at 1:2

Cells were transfected using the mixture of DNA and PEI per industry standard. Cells were fed with one of the 13 feed prototypes (A to M) at 24 h post-transfection using 12% v/v of the culture. The cells were harvested 72 h post-transfection and after the cell lysis used for viral genome and capsid titer assays.

Cell health indicators were monitored before transfection and at the time of harvest.

EXPERIMENTS AND RESULTS

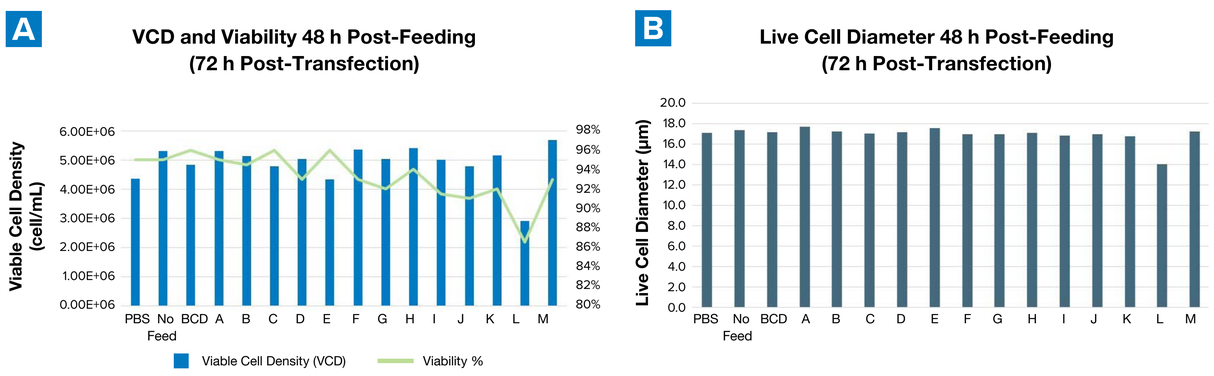

Figure 1. A day following transfection in the shake flasks, HEK293 cells were split into 16 wells in a 24-deepwell plate and fed using either PBS, no feed, or BalanCD HEK293 (BCD, FUJIFILM Irvine Scientific) as controls or one of 13 prototypes (A-M) at 12% v/v at 24 h post transfection. **(A)** VCD and viability were recorded at time of harvest as well as **(B)** cell diameter, indicating that all of the feed media support cell health parameters for the duration of cell culture upon production of AAV2. The data showed that applying the feed in this panel did not negatively impact the cells, and all cell health indices including Viable Cell Density (VCD), Viability, and Cell Diameter are comparable to the "No feed" control.

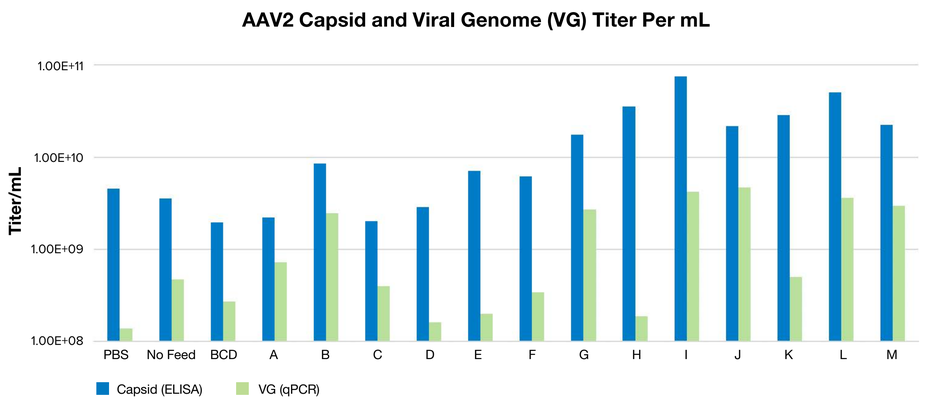

Figure 2. Cell extraction was carried out using three cycles of freeze-thaw. Extract was clarified by centrifugation and treated with DNase I and lysis buffer according to Takara Bio AAV real-time PCR kit instruction prior to qPCR. Green bars indicate the VG titer per mL of the culture, whereas the blue bars indicate the capsid titer per mL measured by Progen AAV2 titration ELISA kit. Feed J is the leading performer and enhances the AAV2 titer approximately 10-fold compared to the "No feed" control.

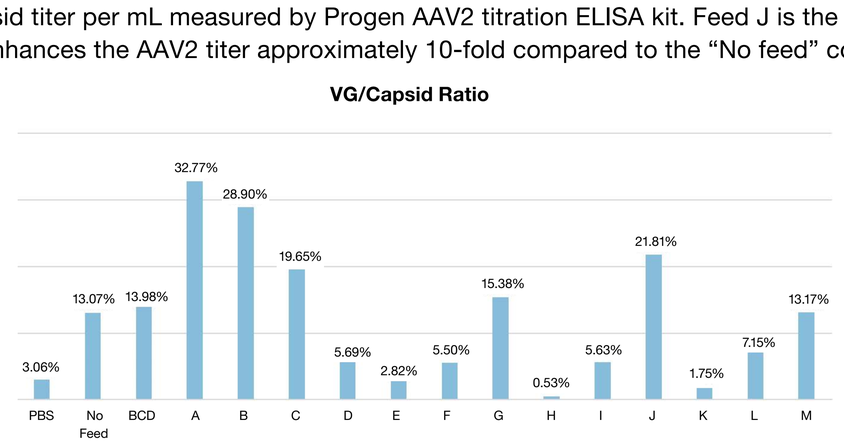

Figure 3. Viral genome (VG) per capsid ratio; an indication of viral packaging efficiency. Feed A packaging efficiency is about 33% followed by Feed B (approx. 29%) and Feed J (approx. 22%).

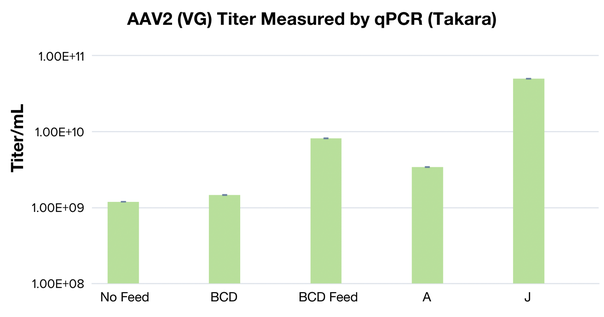

Figure 4. Verification experiment in 30 mL shake flask; AAV2 (VG) titer improved about 10-fold upon applying Feed J compared to "No feed" control.

Selection of the Top-Performing Media Prototypes

Rank			
Feed Type	Titer	VG/capsid	Total
J	1	3	4
B	6	2	8
A	7	1	8
G	5	5	10
M	4	7	11
L	3	9	12
I	2	11	13
C	10	4	14
No Feed	9	8	17
BCD	12	6	18
K	8	15	23
F	11	12	23
D	16	10	26
E	13	14	27
PBS	16	13	29
H	14	16	30

X Times Improvement of Each Prototype Over No Feed Control			
Feed Type	VG	Capsid	Vg/capsid
A	1.55	0.62	2.51
B	5.29	2.39	2.21
J	10.05	6.02	1.67

Fold Improvement Over No Feed Control

Lower Titer — Titer remains the same — Higher Titer

Figure 5. Top-performing media were identified factoring the VG titer and VG per capsid ratio. Feed J enhances AAV production by approximately one log (10X) while improving viral packaging (1.67X). Feed A achieved the most efficient packaging compared to the other prototypes.

FORMULA OPTIMIZATION

Component	% in the Pellet Compared to the Fresh Media
Component 1	73.46
Component 2	0.03
Component 3	0.03
Component 4	0.02

Figure 6. HPLC analysis revealed that the majority (73.46%) of the precipitate is composed of component 1.

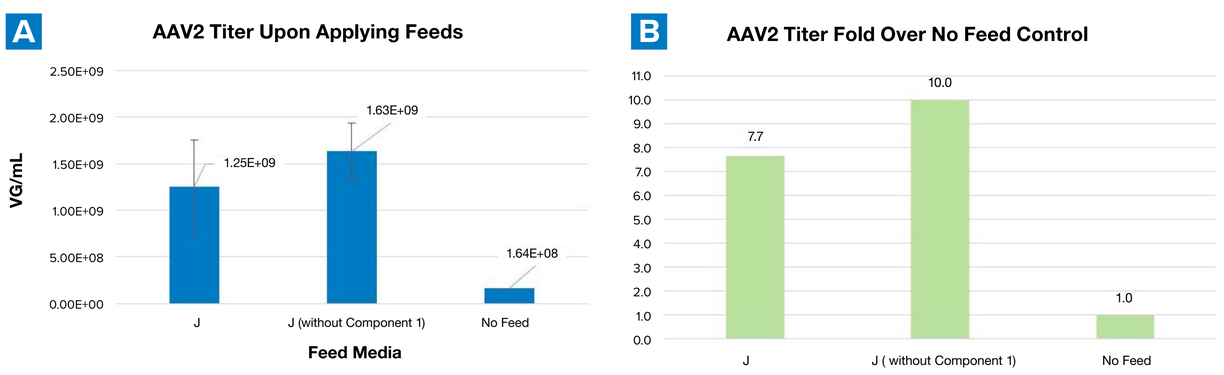

Figure 7. Removing Component 1 from the Feed J formula increases AAV2 titer. The experiment was carried out in 30 mL scale shake flask. Titration study on component 1 concentration in media J revealed that it can be safely removed from the formula without compromising on the media performance.

SUMMARY

- We have developed a feed medium that can boost the production of AAV by about 10-fold and improve the virus packaging efficiency 67% without compromising cell growth and health
- Stability studies and reconfiguration of the formula yielded a feed that is stable in liquid form
- Verification of the results on other AAV serotypes and in higher-scales volumes are in progress