Boosting viral vector production in HEK293 cells using Recombinant Insulin

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Introduction

Viruses have an innate ability to deliver genetic material into cells while avoiding being genetically modified. This attribute has been harnessed by the pharmaceutical industry to develop innovative **vaccine strategies** and **advance cell and gene therapies.** The advancement in this field is heavily reliant on production scale-up and **increased yields.** In this regard, media optimization is fundamental. The **importance of insulin** in serum-free media to promote cell growth and productivity is widely acknowledged.

In this study we examine how the optimization of an in-house developed chemically-defined cell culture media with **Recombinant Insulin** impacts Adenovirus-associated virus **(AAV)** and Lentivirus **(LV)** production in HEK293 cells.

Study description

To establish a HEK293 cell culture with high-yield performance, we evaluated a range of insulin concentrations added at different addition times to maximize cell growth and productivity. In short, suspension-adapted HEK293 cells were cultivated using an in-house developed chemically defined media. Insulin (0, 5, 10, and 20 mg/L) was added 2 hours before transfection, during transfection, or 4 hours after transfection. Production of LV and three commonly used AAV serotypes (AAV-2, AAV-5, and AAV-8) was evaluated according to the protocol outline in **Fig. 1.**

Seed	Transfection	Growth	Harvest	Infection	Titer
Seed HEK293 cells in 120 mL shake flask	Transfect HEK293 with a LV four plasmid system containing a GFP tag using PEI	Follow growth and viability for 72 hour after transfection	After 48h, harvest the LV particles by collecting the supernatant	Infect HT1080 cells with LV containing supernatant	After 72h, target gene (GFP) detected by FACS method (TU/ml)
Seed	**Transfection**	**Growth**	**Harvest**	**ITR detection**	**Titer**
Seed HEK293 cells in 120 mL shake flask	Transfect HEK293 with an AAV three plasmid system using PEI	Follow growth and viability for 72 hour after transfection	Harvest AAV containing supernatant at 72h post- transfection	DNA was extracted and the target gene (ITR) detected by qPCR	ITR content was used to calculate viral titer (VG/ml)

Figure 1 | Schematic overview of workflow for LV and AAV production and quantification. The protocol for LV production is shown in the top panel. The bottom panel illustrates the workflow for AAV production.

Insulin enhances LV production > 2-fold

Insulin boosted cell proliferation of LV-producing HEK293 cells, with the most significant impact seen when 10 mg/L of insulin was added 2 hours before transfection **(Fig. 2A)**. Additionally, addition of 20 mg/L insulin 2 hours before transfection resulted in a 110% increase in LV titer **(Fig. 2B)**.

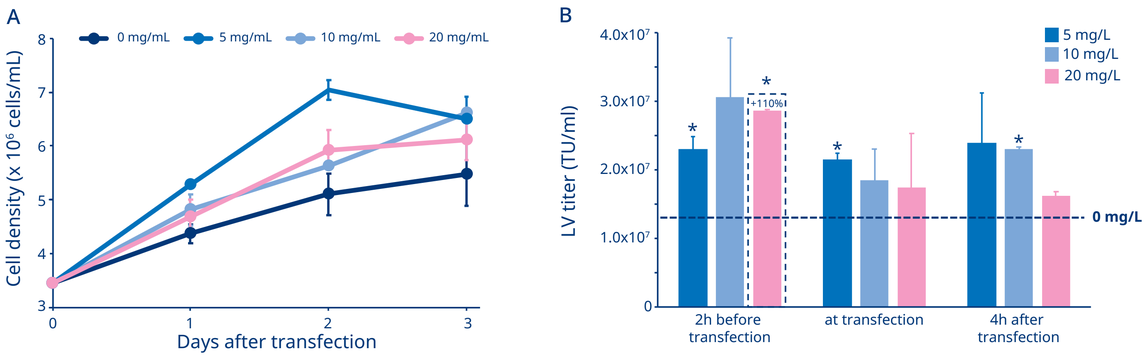

Figure 2 | Addition of insulin 2 hours before transfection improves lentivirus titers by 110%. (A) Cell density of LV-producing HEK293 cells following addition of insulin 2 hours before transfection. (B) Transduction units per mL (TU/mL) following the addition of Recombinant Insulin (5, 10, or 20 mg/L) 2 hour before transfection, at transfection, or 4 hours after transfection. The dashed line represents the baseline for the untreated control (0 mg/L insulin).
* P < 0.05; statistical analysis was performed using an unpaired t-test.

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Insulin boosts AAV production by > 40%

Insulin did not impact cell proliferation of AAV-producing HEK293 cells regardless of addition time and concentration (Fig. 2A, C, E). Despite an absence of increased cell density, insulin increased viral titers significantly (Fig. 2B, D, F). Specifically, adding 10 mg/L insulin 2 hours before transfection increased the AAV2 titer by **47%** (Fig 2B), the AAV5 titer by **40%**, and the AAV8 titer by **44%.**

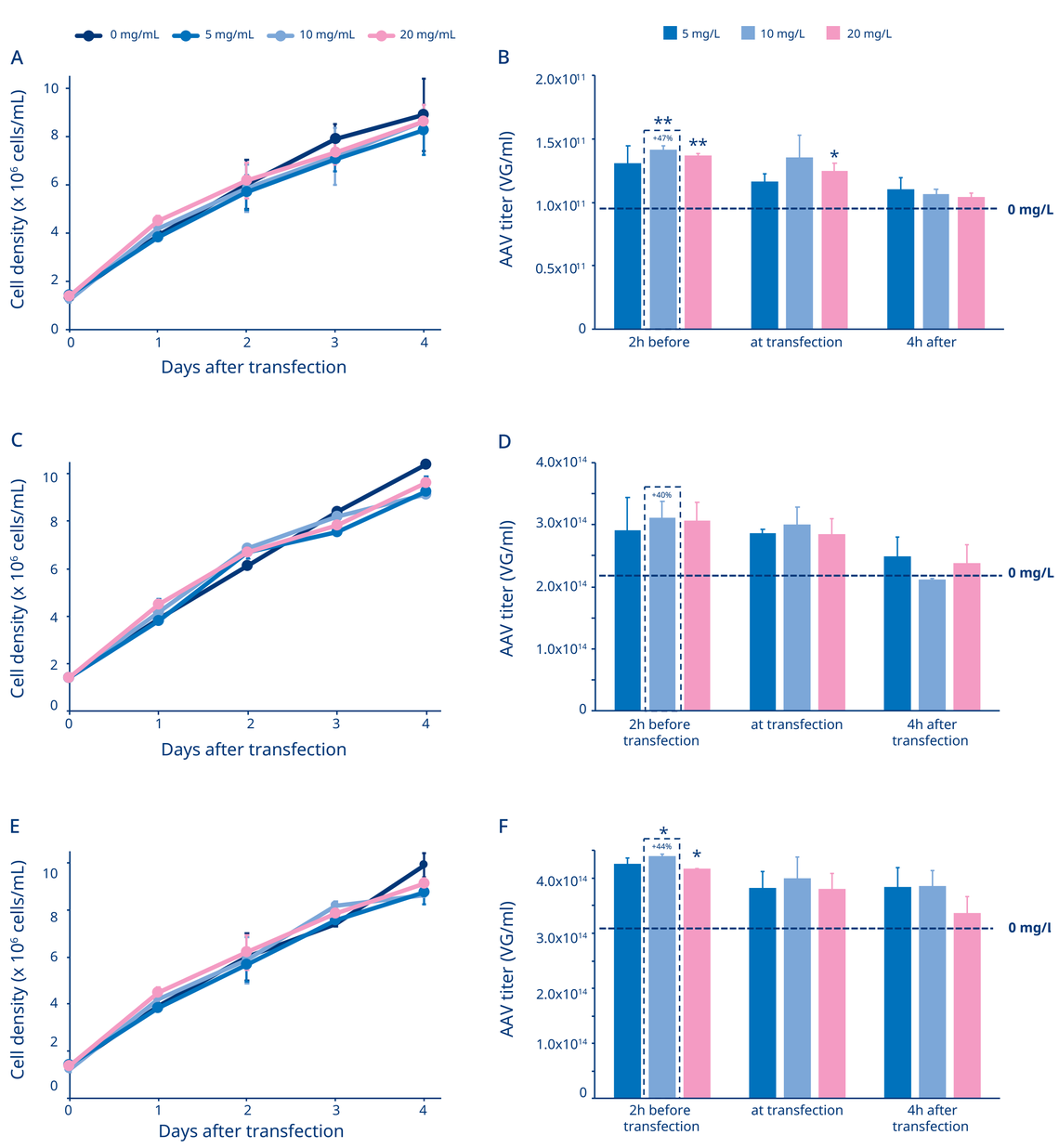

Figure 3 | Addition of insulin 2 hours before transfection improves AAV titers by more than 40%. Cells density of HEK293 cells expressing AAV2 (A), AAV5 (C), and AAV8 (E) particles following addition of insulin 2 hours before transfection. For full data set use QR code below. AAV titers per mL (VG/mL) following the addition of Recombinant Insulin (5, 10, or 20 mg/L) 2 hour before transfection, at transfection or 4 hours after transfection for AAV2 (B), AAV5 (D), and AAV8 (F). The dashed line represents the baseline for the untreated control (0 mg/L insulin). * P < 0.05, ** P < 0.01; statistical analysis was performed using an unpaired t-test.

Conclusions

LV titers were boosted by up to 110% with recombinant insulin. The highest titers were achieved by adding 20 mg/l of recombinant insulin to cell culture media 2 hours prior to transfection.

AAV production was increased by up to 47% with the addition of Recombinant Insulin. All AAV serotypes tested increased their titers by more than 40%, without affecting cell proliferation, which indicates increased productivity in the presence of insulin.

Adding Recombinant Insulin to chemically defined cell culture media could offer significant cost savings via increased viral titers in AAV and LV production.

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