Production of serotype 6-derived recombinant adeno-associated virus in serum-free suspension cultures of HEK 293 cells

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INTRODUCTION

- Recombinant adeno-associated viruses (rAAV) ⇨ promising gene therapy candidate vectors
- Key advantages of rAAV vectors ⇨ good safety profile and broad tissue tropism through the use of different serotypes
- Most common approach for rAAV production ⇨ transient transfection of mammalian cell cultures
- HEK 293 (human embryonic kidney cells) ⇨ cell line used for production of a licensed recombinant protein and largely studied for the production of vaccines and gene therapy vectors (specially adenoviral and adeno-associated viral vectors)
- HEK 293 advantages ⇨ relatively easy adaptation to suspension cultivation in serum- and animal-derived-component-free media (complying with regulatory needs) and high transfection efficiencies with most gene transfer vehicles

OBJECTIVE OF THE WORK

- To investigate the effects of different cell densities, DNA and transfection reagent (PEI) concentrations on rAAV yields

METHODOLOGY

- HEK 293-SF-3F6 cells ⇨ suspension cultivation, using 125-mL shake flasks (20-mL working volume) or stirred-tank bioreactors (2-L working volume)
- Culture medium ⇨ serum-free SFM4 TransFx-293 (Hyclone) with 0.1% Pluronic F68 (Sigma-Aldrich)
- Cell concentration at transfection: 1, 1.5, 2 and 3 million cells/mL (Figure 1) or 1 million cells/mL (Figures 2 and 3)
- Transfection reagent ⇨ 25-kDa linear polyethylenimine (PEI) (Polysciences)
- Three plasmids were used, at a molar ratio of 1:1:1 :
  - Plasmid ssITRCMV-EGFP, containing the EGFP gene flanked by the AAV ITR sequences (single-stranded DNA)
  - Rep/Cap plasmid containing genes associated to replication and capsid-forming proteins
  - Helper plasmid containing adenovirus genes required by AAV
- Total plasmid DNA load: 1 µg of plasmid DNA per mL of cell culture
- Responses ⇨ IVP (infectious virus particles) and Vg (genomic particles)

RESULTS

Evaluation of the effects of different DNA, PEI and cell concentrations on the production of AAV

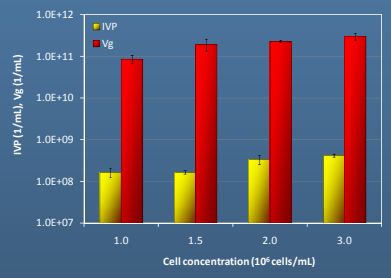

Figure 1 – IVP and Vg titers obtained 48 h post-transfection, with different cell concentrations at moment of transfection (1, 1.5, 2 and 3 million cells/mL). Transfection conditions: 1 µg of total plasmid DNA per mL of cell culture, 2 µg of PEI per mL of cell culture, PEI-to-DNA ratio of 2.0.

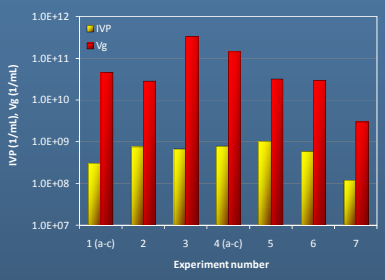

Figure 2 - IVP and Vg titers obtained 48 h post-transfection in experiments # 1-7, where different DNA and PEI concentrations have been evaluated (see Table 1 for DNA and PEI values). Cell concentration at transfection: 1 million cells/mL.

Table 1 – DNA and PEI concentrations and PEI-to-DNA ratios in experiments of Figure 2.

Exp. #	DNA (µg/mL)	PEI (µg/mL)	PEI-to-DNA ratio
1	0.2	4.00	20.00
2	0.2	2.75	13.75
3	0.6	2.75	4.58
4	0.6	2.50	4.17
5	0.6	1.60	2.67
6	1.0	1.80	1.80
7	1.0	1.20	1.20

Evaluation of process scalability to stirred-tank bioreactors

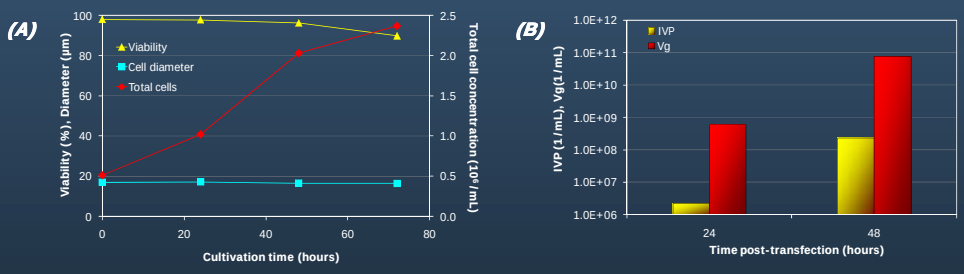

Figure 3 – rAAV production in a stirred-tank bioreactor with 2-L working volume. Transfection was done at 24 h and 1x10⁶ cells/mL, using 1.6 µg/mL PEI and 0.6 µg/mL DNA (PEI:DNA = 2.67). (A) Kinetics of cell growth, showing total cell concentration, cell viability and cell diameter from time of inoculation (0 h) up to 48 h post-transfection. (B) IVP and Vg titers 24 h and 48 h post-transfection.

CONCLUSIONS

- rAAV production was slightly affected by cell concentration at time of transfection, since transfection at 3x10⁶ cells/mL resulted in a 2.5- and 3.5-fold increase in IVP and Vg, respectively, when compared to transfection at 1x10⁶ cells/mL.
- When different PEI:DNA ratios were tested, IVP varied from 1.19x10⁸ IVP/mL to 1.03x10⁹ IVP/mL, whereas Vg varied from 3.03x10⁹ Vg/mL to 3.34x10¹¹ Vg/mL. The results indicate that the individual concentrations of PEI and DNA have a more pronounced effect on IVP and Vg titres than the ratio between both of them.
- In stirred-tank bioreactors, at 48 hours post-transfection, IVP and Vg values were higher than 2x10⁸ IVP/mL and 7x10¹⁰ Vg/mL, respectively. Adopting the methodology used in this work, assuming that similar virus titers can be obtained upon further scale-up of the process, a 1000-L bioreactor could produce a total amount of approximately 1x10¹⁴ IVP and 1x10¹⁶ Vg particles. Assuming an overall downstream processing recovery of 50%, these quantities should be sufficient for use in large clinical trials.

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