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

Érica A. Schulze¹,², Parminder Chahal³, Ricardo A. Medronho¹, Leda R. Castilho², Júlio Fernandes³, Amine Kamen³

¹Federal University of Rio de Janeiro, School of Chemistry, CT, Bl. E, 21941-909 Rio de Janeiro/RJ, Brazil
²Federal University of Rio de Janeiro, CNPPE, Cell Culture Engineering Lab, Caixa Postal 68502, 21941-972 Rio de Janeiro/RJ, Brazil
³Biotechnology Research Institute, National Research Council, 5100 Royalment Avenue, HP 292, Montreal, QC, Canada

INTRODUCTION

Recombinant adeno-associated viruses (rAAV) are promising gene therapy candidate vectors. Key advantages of rAAV vectors include good safety profile and broad tissue tropism through the use of different serotypes. Most common approach for rAAV production is transient transfection of mammalian cell cultures. HEK 293 (human embryonic kidney cells) cell line was used for production of a licensed recombinant protein and is largely studied for the production of vaccines and gene therapy vectors (speciality adeno and adeno-associated viral vectors).

HEK 293 advantages include 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 plasmid concentrations, DNA, and transfection reagent (PEI) concentrations on rAAV yields.

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 DNA 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.

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

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

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.

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 = 2.8:1). (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 1.19x10⁴ to 1.03x10⁵ IVP/mL to 1.03x10⁵ IVP/mL, whereas Vg varied from 3.03x10⁸ 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 titers than the ratio between both of them.
- In stirred-tank bioreactors, at 48 hours post-transfection, IVP and Vg values were higher than 2x10⁶ IVP/mL and 7x10⁸ 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 1x10¹⁵ Vg particles. Assuming an overall downstream processing recovery of 50%, these quantities should be sufficient for use in large clinical trials.

ACKNOWLEDGEMENTS: Université de Montréal, BCEI / MABCI, NRC-CNRC and BII & Melinda Gates Foundation