Adenovirus production in a single-use stirred-tank bioreactor system

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Introduction
Adenovirus vectors are attractive delivery systems for vaccines and cancer treatment. Scalable and cost-efficient production technologies are needed to enable manufacturing of safe and efficacious clinical-grade virus. Anchorage-dependent cells cultured in roller bottles or cell factories are commonly used in these processes. However, scale-up using these techniques is complicated and limited by the surface available for cell growth. One alternative is to scale up the production on microcarriers. Another solution is to use suspension-adapted cells, which can facilitate scale-up possibilities.

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Infectious virus titer (ivp/mL)

1.0E+06
1.0E+08
1.0E+10

6.0
0.0
1.0
2.0

7.0 100

0 1 2 3 4 5 6

MOI 1 (r1)

MOI 1 (r2)

MOI 10 (r1)

MOI 10 (r2)

Fig 1. Infectious virus titer determined using automated fluorescence microscope counting GFP foci.

Materials and methods
• Cell lines: HEK-293.2 (ATCC), adapted to the different CCM
• Virus: recombinant adenovirus serotype 5 expressing GFP (Ad5-GFP)

CCM 1: commercially available serum-free CCM (all developed for HEK293 cells)

Small-scale vessels: 125–500 mL

Large-scale vessels: Xcellerex XDR-10 Bioreactor

Infectious virus particles (ivp) was determined with an automated fluorescence microscope counting GFP foci.

Total virus particles (vp) was determined with qPCR.

Optimization in small scale
Cell growth in batch mode was evaluated in five different CCM (named A–E) all developed for HEK293 cells. The capacity of the different CCM varied with regards to cell growth and viability (Fig 2). Only medium A and B were able to support a cell density above 2 × 10^6 cells/mL. Cells were also monitored by microscopy (Fig 3) to follow morphology and aggregate formation. CCM B and E were the two media that best supported cell growth and formed fewer aggregates.

Ad5-GFP productivity was evaluated for CCM A and B, with multiplicity of infection (MOI) of 1 and 10. The infectious virus titer was higher for medium B in all experiments (Fig 4). Therefore, medium B was used in further optimization. Medium B is CDM4GEK293 (HyClone™, GE Healthcare). The effect of different MOI was further investigated with a range of MOI from 0.1 to 10 (Fig 5). Between MOI 2 and 10 there was a slight increase in productivity that seemed to level off at higher MOI. MOI 10 was chosen for the scale-up process due to the presence of a high cell density, data not shown.

Scale-up in Xcellerex XDR-10 bioreactor
The Xcellerex XDR-10 bioreactor was inoculated at 0.3 × 10^6 cells/mL. Cells were grown in the bioreactor at 48 h post-infection (dpi). Reactor settings and process parameters are listed in Table 1. Two shake flask controls were included for comparison of cell growth and viability (Fig 6). Infectious virus titer did not increase significantly between 42 and 48 hpi (Fig 6). Ad5-GFP was chosen for future harvest because of a more efficient and convenient process. Figure 7 also shows that infectious virus titer is not affected by detergent cell lysis. The ratio between total virus particles and infectious virus titer at 42 hpi in the bioreactor is approximately 15:1, which is within regulatory standards for Ad5 (Fig 8). Both cell growth and virus productivity were improved in the bioreactor scale-up as compared to shake flask cultures.

Conclusions
• Screening and selection of cell culture media is important for both cell growth and virus productivity.
• The results indicate robust scalability of shake flasks and the Xcellerex XDR bioreactor format.
• Debundled efficient scale-up of Ad5-GFP production in Xcellerex XDR-10 bioreactor with a final titer of 10^10 vp/mL in batch culture.

Table 1. Bioreactor settings and process parameters for virus titer analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO setpoint</td>
<td>40%</td>
</tr>
<tr>
<td>DOG</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 °C</td>
</tr>
<tr>
<td>agitation</td>
<td>100 µm</td>
</tr>
<tr>
<td>Sparger</td>
<td>Air, CO₂ 0.5 mm</td>
</tr>
<tr>
<td>Sparger 2</td>
<td>Air, O₂ 20 µm</td>
</tr>
<tr>
<td>TDI</td>
<td>1 x 10^8 cells/L</td>
</tr>
<tr>
<td>MDI</td>
<td>10</td>
</tr>
<tr>
<td>TDI</td>
<td>48 h</td>
</tr>
</tbody>
</table>

Fig 2. Infectious virus titer (ivp/mL) and infectious virus titer (vp/mL) for different CCM (A–E).

Fig 6. Infectious virus titer (ivp/mL) and infectious virus titer (vp/mL) for virus titer analysis.

Fig 8. Infectious virus titer (ivp/mL) and infectious virus titer (vp/mL) for virus titer analysis.

Fig 7. Infectious virus titer determined using automated fluorescence microscope counting GFP foci.