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

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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.

In this work we demonstrate an efficient process for adenovirus production in a single-use stirred-tank bioreactor, using HEK293 cells adapted for suspension culture. Human adenovirus 5 expressing green fluorescent protein (GFP) was used as a model system. By evaluating different cell culture media (CCM) and optimizing the virus propagation in small scale, the process could be successfully established in Xcellerex™ XDR-10 Bioreactor (10 L working volume). This process opens up possibilities for further scale-up to production scale. Figure 1 presents the strategy for this study.

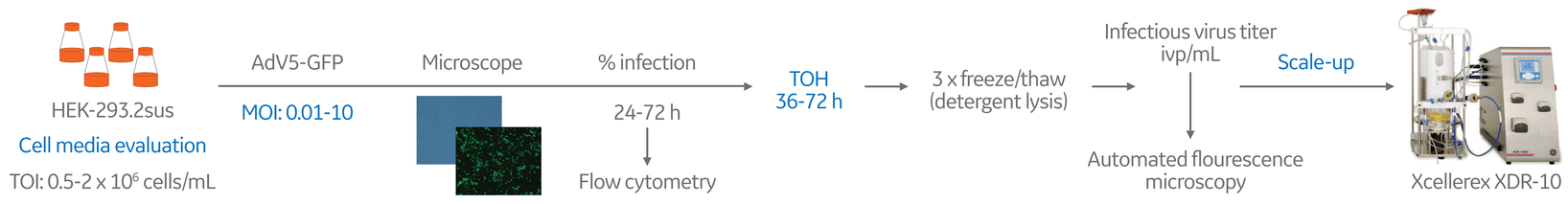

Fig 1. Figure illustrating the process development strategy used for the study. TOI = time of infection; MOI = multiplicity of infection; TOH = time of harvest.

Materials and methods

- Cell line: HEK-293.2sus (ATCC), adapted to the different CCM
- Virus: recombinant adenovirus serotype 5 expressing GFP (AdV5-GFP)
- CCM: 5 commercially available serum-free CCM, (all developed for HEK293 cells)
- Small-scale vessels: 125–500 mL Erlenmeyer shake flasks
- Large-scale vessel: Xcellerex XDR-10 Bioreactor
- Infectious virus particles (ivp) was determined with an automated fluorescent 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 media B and E were able to support a cell density above 2.5 × 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.

AdV5-GFP productivity was evaluated for CCM B and E, 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 CDM4HEK293 (HyClone™, GE Healthcare). The effect of different MOI was further investigated with a range of MOI from 0.01–10 (Fig 5). Between MOI 1 and 2 there was a slight increase in productivity that seemed to level off at higher MOI. MOI 10 was chosen to ensure the higher titer seen at MOI 2 and higher. Time of infection (TOI) of 1 × 10^6 cells/mL was chosen for the scale-up process due to loss of ivp/cell at higher cell densities, data not shown.

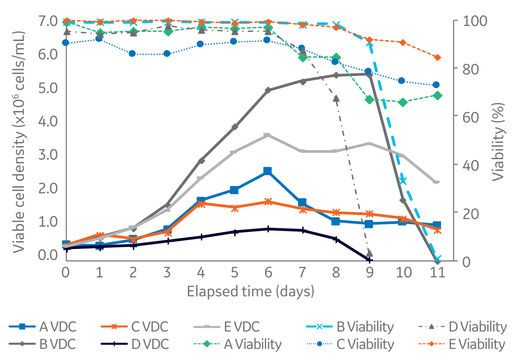

Fig 2. Cell growth and viability in CCM evaluation. VCD = viable cell density.

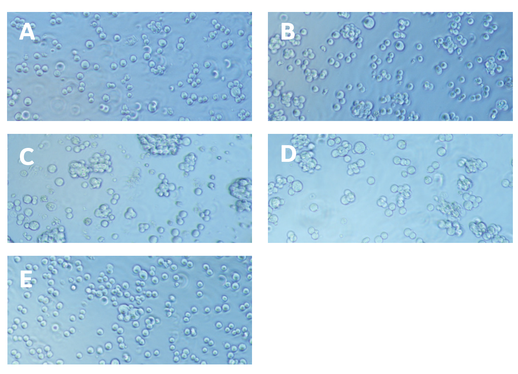

Fig 3. Photos of cells grown in different CCM (A-E).

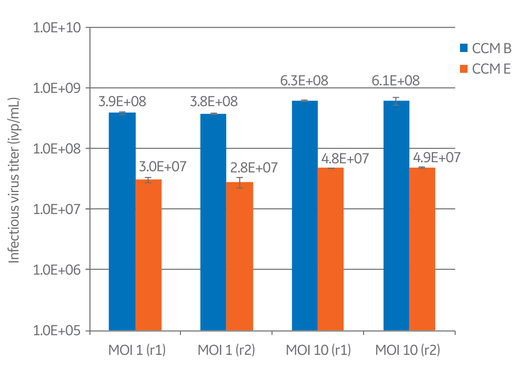

Fig 4. Infectious virus titer in batch cultures grown in media B and E with MOI 1 and 10. At TOI the cultures were diluted with fresh cell culture media. TOI: 1 × 10^6 cells/mL; TOH: 42 h. Each culture was performed in two replicates (r1, r2).

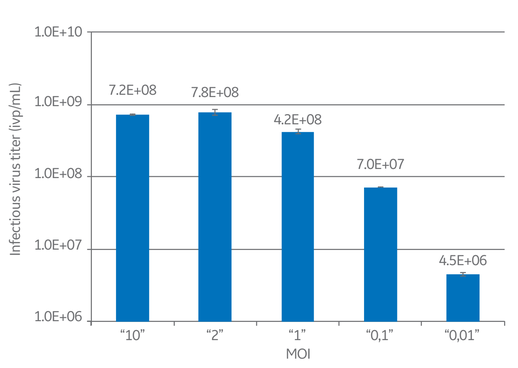

Fig 5. Infectious virus titer with different MOI at 42 h post-infection. Cells were grown in medium B (CDM4HEK293) and diluted with the same medium before infection at 1 x 10^6 cells/mL.

Scale-up in Xcellerex XDR-10 bioreactor

The Xcellerex XDR-10 bioreactor was inoculated at 0.3 × 10^6 cells/mL. Cells were grown to 2 × 10^6 cells/mL in CDM4HEK293 medium, and then a two fold dilution was performed. At 1 × 10^6 cells/mL the cells were infected using a MOI of 10. Cell lysis was performed in the bioreactor at 48 h post-infection (hpi). 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 7). 42 hpi 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 AdV (Fig 8). Both cell growth and virus productivity were improved in the bioreactor scale-up as compared to shake flask cultures.

Table 1. Bioreactor settings and process parameters for culture in Xcellerex XDR-10 bioreactor

Bioreactor settings and process		
Cell line	HEK293.2 sus	
Cell culture media	CDM4HEK293	
Additions	4mM L-gln	
Starting cell density	0.3 x 10^6 cells/mL	
Starting volume	5 L	
Final volume	10 L	
pH setpoint	7.1	
DO setpoint	40%	
Temperature	37 °C	
Agitation	100 rpm	
Sparger	Air, CO_2	0.5 mm
Sparger 2	Air, O_2	20 µm
TOI	1 x 10^6 cells/mL	
MOI	10	
TOH	48 h	

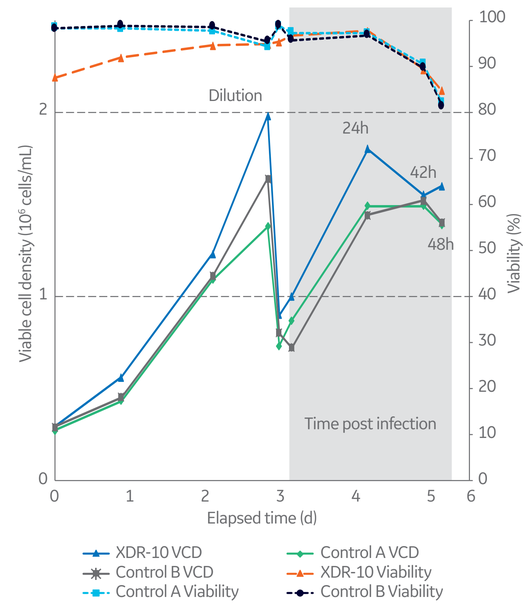

Fig 6. Cell growth and viability – comparison between bioreactor culture and shake flask cultures (control A and B). Samples were withdrawn at 24, 42 and 48 h post-infection for virus titer analysis.

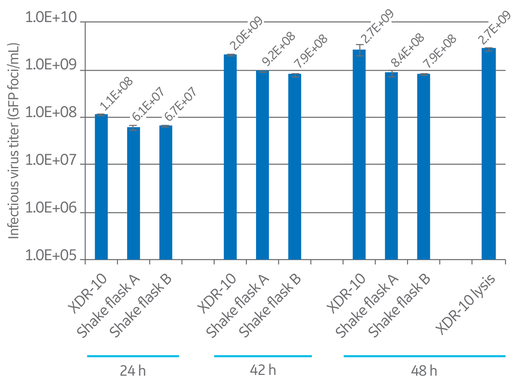

Fig 7. Infectious virus titer determined using automated fluorescence microscopy at 24, 42, and 48 hpi in Xcellerex XDR-10 bioreactor and controls.

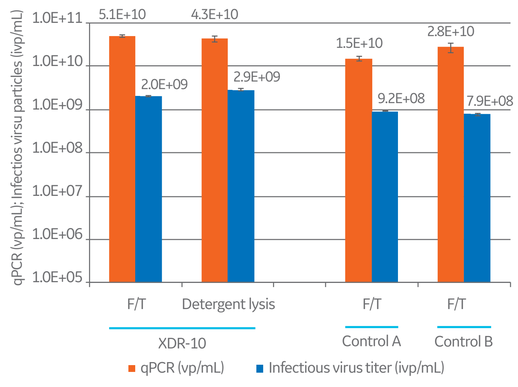

Fig 8. Total virus particles (vp/mL) and Infectious virus titer at 42 h post-infection.

Conclusions

- Screening and selection of cell culture media is important for both cell growth and virus productivity.
- The results indicate robust scalability between shake flasks and the Xcellerex XDR bioreactor format.
- We demonstrated efficient scale-up of AdV5-GFP production in Xcellerex XDR-10 bioreactor with a final titer of 10^9 ivp/mL in batch culture.

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