Adenovirus production in single-use ReadyToProcess WAVE 25 bioreactor system

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Adenovirus production in single-use ReadyToProcess WAVE™ 25 bioreactor system

This application note describes the production of adenovirus in HEK293 suspension cells grown in HyClone™ CDM4HEK293 culture medium using the ReadyToProcess WAVE 25 bioreactor system. Triplicate cultures demonstrate a reproducible and robust batch production of adenovirus. Cell culture was conducted in Cellbag™ bioreactors manufactured from either BioClear™ 11 or Fortem™ films.

Introduction

Adenovirus is one of the most frequently used viral vectors in the development of therapeutic vaccines and vaccines against infectious diseases (1). Adenovirus has also been explored as a viral vector for gene therapy and as an oncolytic virus. Several generations of recombinant adenovirus vectors have been developed to enhance productivity and to produce more effective and safer vaccines (2). One of the most studied adenovirus vector is the first generation of recombinant adenovirus serotype 5 (AdV5), making this a suitable system for development of a process for adenovirus production.

Typically, upstream virus production for vaccine manufacturing is performed in stirred-tank bioreactor systems. However, rocking bioreactor systems offer the benefits of low minimum working volumes that can shorten the seed train prior to inoculation, and agitation can be adjusted to a smooth rocking motion for optimized growth of sensitive cells.

Human embryonic kidney 293 (HEK293) cells are susceptible to infection by adenovirus, and are commonly used in adenovirus, production. Previous work has identified CDM4HEK293 culture medium as a good choice for efficient HEK293 cell growth and adenovirus productivity (3), and a complete upstream process was established using the single-use Xcellerex™ XDR-10 stirred-tank bioreactor system (4). This work evaluates adenovirus production in HEK293 cells, using the ReadyToProcess WAVE 25 rocking bioreactor system. Cell growth and virus productivity were also compared between parallel cultures conducted in disposable Cellbag bioreactor bags manufactured from either BioClear 11 film or Fortem film (5).

Materials and methods

Bioreactor cultures

A 20 L Cellbag bioreactor was inflated with air and 1 L of CDM4HEK293 medium was added aseptically to the bag. The bioreactor bag was left for equilibration over night at 37°C, in 5% CO_2, at an agitation of 12 rpm. Before cell inoculation, the agitation was increased to 20 rpm and an offset calibration of pH 7.1 was performed.

HEK293.2sus cells, expanded in shake flask cultures in CDM4HEK293 cell culture medium supplemented with 4 mM L-glutamine, were inoculated at a cell density of approximately 0.3×10^6 cells/mL, reaching a starting volume of 1.5 L in the bioreactor bag. After three days, the culture was diluted in prewarmed complete CDM4HEK293 medium to a cell density of 0.3×10^6 cells/mL in a volume of 5–6 L, and agitation was increased to 23 rpm. After three more days and prior to adenovirus infection, the culture was diluted in a similar manner to approximately 1.0×10^6 cells/mL in an operating volume of 6–10 L, and the rocking was increased to 25 rpm. Culture parameters are listed in Table 1.

Shake flask control cultures

HEK293.2sus cells were expanded in complete CDM4HEK293 cell culture medium in shake flasks. For each bioreactor culture, one culture was performed in parallel in a shake flask, using culture parameters listed in Table 2 , as control for cell morphology and virus productivity.

Virus propagation

AdV5 production was performed in both bioreactor and shake flask control cultures. Cell cultures were infected with E1/E3-deleted AdV5, coding for green fluorescent protein (GFP), at a multiplicity of infection (MOI) of 10 and a time of infection (TOI) of $0.9–1.1 \times 10^6$ cells/mL. Time of harvest (TOH) was 42 h post infection, and virus was released in the bioreactor by addition of 0.5% Tween™ 20 (6). Acceptance criteria for this study were set to infectious virus particles of $\geq 10^9$ ivp/mL. Furthermore, target infectivity ratio (total-to-infectious virus particles [tvp:ivp]) was set to ≤ 30:1.

Table 1. Parameters used for bioreactor cultures

Production medium	CDM4HEK293 supplemented with 4 mM L-glutamine
Starting viable cell density	0.3 × 10^6 viable cells/mL
Starting volume	1–1.5 L
Operating volume	1–10 L
Agitation	12–25 rpm
Rocking motion	30%
Angle	7°
Temperature	37°C
Gas flow	0.2 L/min
pH set-point	7.1 (controlled by CO_2 and $NaHCO_3$)
Base for pH control	7.5% (w/v) $NaHCO_3$
Dissolved oxygen (DO) set-point	40% (controlled by oxygen-enriched air).

Table 2. Parameters used for shake flask cultures

Production medium	CDM4HEK293 supplemented with 4 mM L-glutamine
Starting viable cell concentration	0.3 × 10^6 viable cells/mL
Starting volume	200 mL
Agitation	100 rpm
Temperature	37°C
CO_2	5%
Humidity	80%

Analysis

The cultures were sampled for analysis of cell growth, viability, morphology, metabolites, nutrient consumption, and pH. Cell growth and viability were determined using the Vi-CELL™ XR analyzer (Beckman Coulter). Glucose, glutamine, glutamate, lactate, and ammonium were measured using the CEDEX™ Bio analyser (Roche). Cell morphology was examined microscopically. Culture pH was determined both online and offline for the bioreactor cultures and offline for the control cultures.

Infectious virus titer in harvest samples was analyzed using adherent HEK293 cells. When cells reached 85%–95% confluence, virus titration of harvested sample was added to the cells, and AdV5-GFP-infected cells were detected and counted after 42 h by automated fluorescence microscopy using the IN Cell Analyzer 2200.

Total virus titer was determined by qPCR. Viral DNA was purified using PureLink™ Viral RNA/DNA Mini Kit (Thermo Fisher Scientific) and quantified by qPCR using specific primers against the adenovirus hexon gene, using Human Adenovirus 5 Wild Type Quantitated DNA (Virapur) as reference material.

Results

Three bioreactor cultures were performed, where Run 1 and 2 were performed with Cellbag bioreactors manufactured from the BioClear 11 film and Run 3 was performed with the bioreactor bag manufactured from Fortem film. The different runs were conducted to evaluate reproducibility between the different cultivations and to compare culture performance of the two different bioreactor bags. Cell morphology and appearance of cell aggregation were monitored daily in microscope during seed train until harvest. Figure 1 shows morphology of cells in the bioreactor cultures as well as in the shake flask control cultures before infection and at TOH.

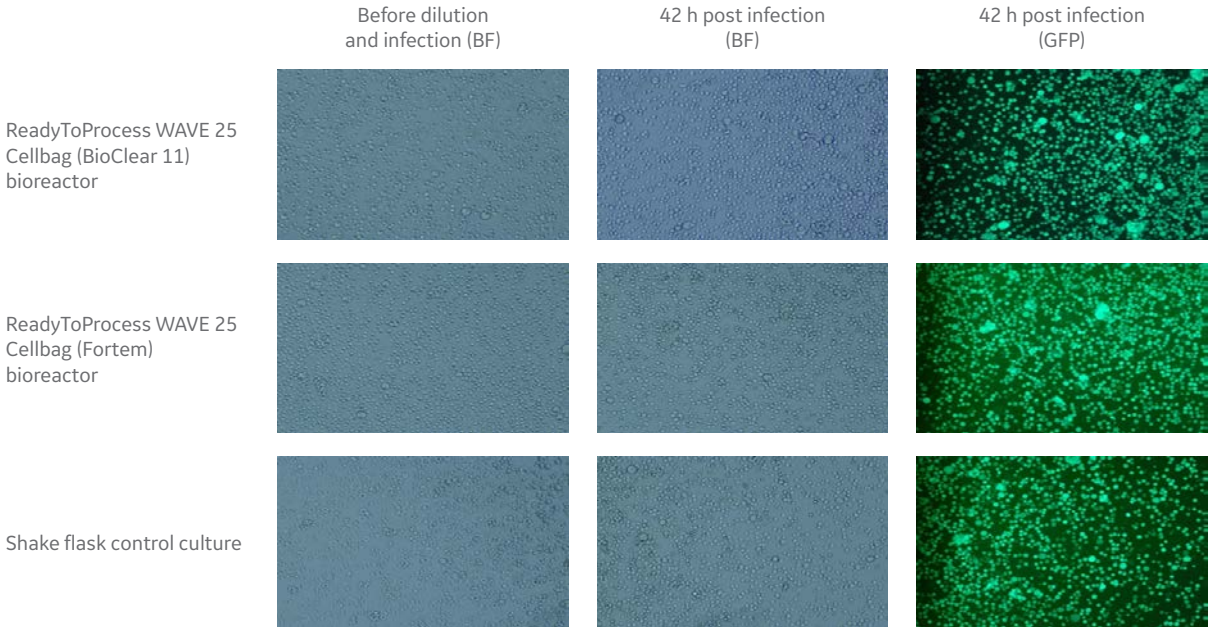

Fig 1. Cell morphology over the culture period. BF = bright field, GFP = green fluorescent protein.

Cell growth and viability were followed carefully during cell expansion and virus infection (Fig 2). Nutrients, metabolites, and pH were monitored during cell expansion until TOI (Fig 3 and 4). Cell growth, viability, and nutrient consumption were similar between the different bioreactor cultures.

Infectious virus titer was determined by detection of GFP expressing cells by automated fluorescence microscopy using the IN Cell Analyzer, and total virus particles were monitored by quantification of hexon DNA by qPCR. Infectious and total virus particles are shown in Figure 5. All bioreactor cultures showed virus titers of ≥ 10^9 ivp/mL. The tvp-to-ivp ratio in the harvested material was below 30:1 for all three cultivations.

No difference in culture performance was observed between the BioClear 11 and Fortem bags. Cell productivity of adenovirus from the bioreactor culture was similar or better to what was achieved in shake flask cultures. The results demonstrate a reproducible and robust batch production process for adenovirus using the ReadyToProcess WAVE 25 bioreactor system in 10 L scale. Results obtained in this study, using the ReadyToProcess WAVE 25 bioreactor system, are also comparable with results obtained in previous work, using the Xcellerex XDR-10 bioreactor system (4).

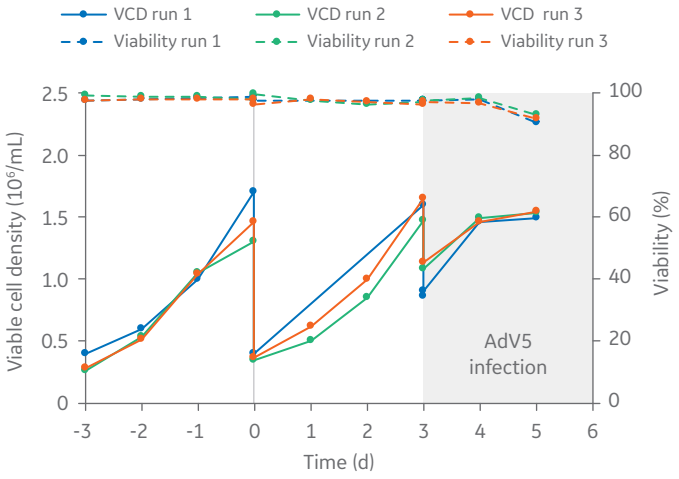

Fig 2. Viable cell density (VCD) and viability in bioreactor Run 1, 2, and 3. Seed train in bioreactor starting at 1.5 L. Cell culture medium was added Day 0 and 3 to reach final bioreactor volume with 1 × 10^6 cells/mL prior to infection with AdV5-GFP at Day 3. Run 1 and 2: Bioclear 11 film, Run 3: Fortem film.

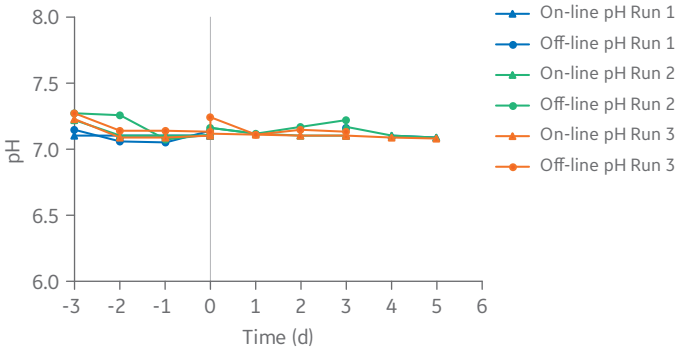

Fig 3. Culture pH measured online (Day -3 to 5) and offline (day -3 to 3).

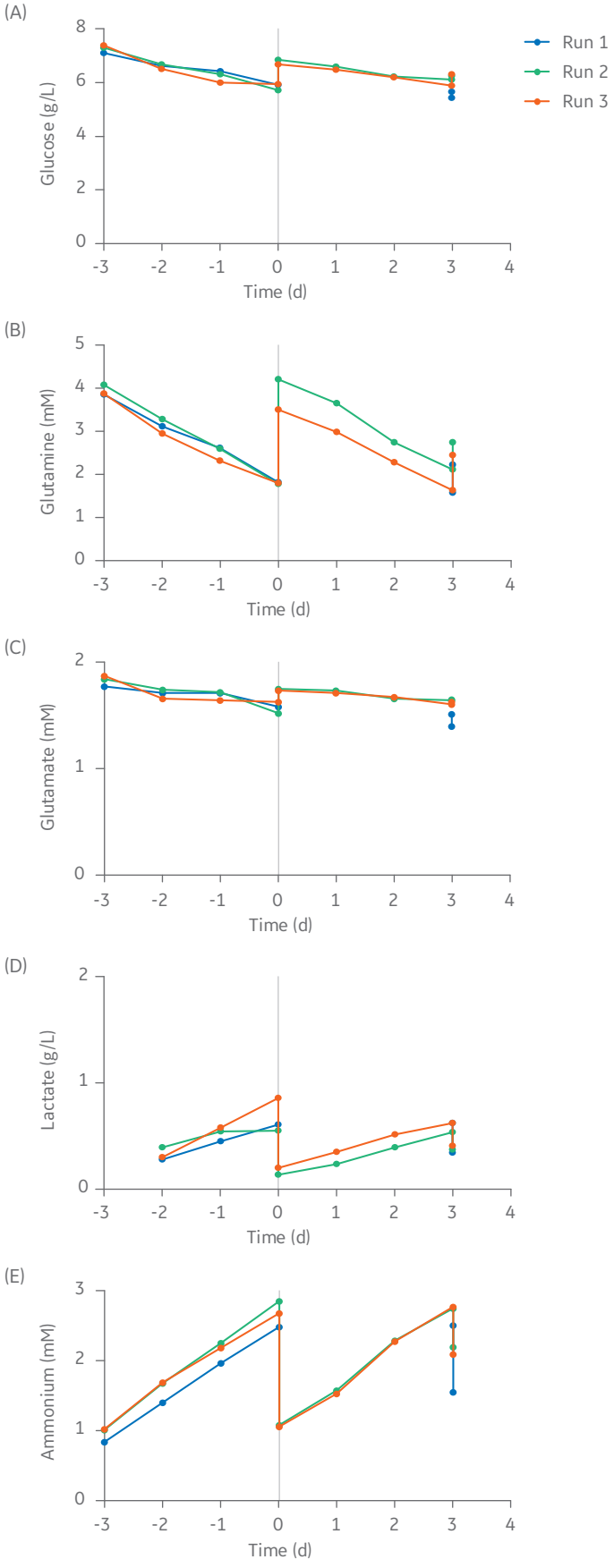

Fig 4. Bioreactor culture concentrations of (A) glucose, (B) glutamine, (C) glutamate, (D) lactate, and (E) ammonium. No metabolites were determined after virus infection (Day 4 and 5).

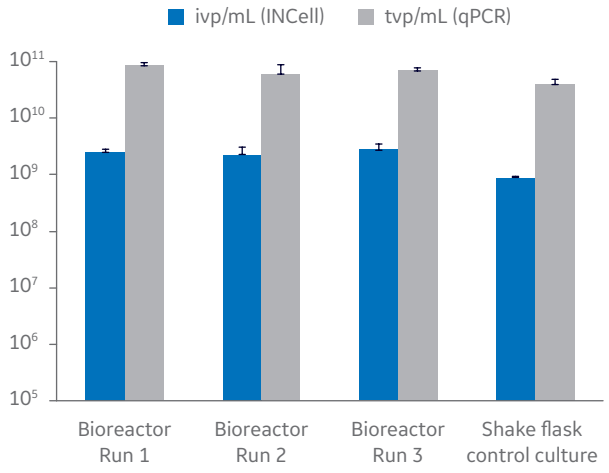

Fig 5. Determination of infectious virus particles (ivp/mL) by automated fluorescence microscopy using the IN Cell Analyzer and total virus particles (tvp/mL) by qPCR in the three bioreactor cultures and one shake flask control culture.

Conclusion

This work demonstrates robust production of adenovirus in HEK293 suspension cells grown in CDM4HEK293 medium using the ReadyToProcess WAVE 25 bioreactor system. Similar cell growth and productivity was achieved in three bioreactor runs, indicating process robustness. No difference in culture performance could be observed between Cellbag bioreactors manufactured from either BioClear 11 or Fortem film. The results in terms of HEK293 cell growth and adenovirus production obtained in ReadyToProcess WAVE 25 are similar to what was previously described for XDR-10, showing robustness between the different bioreactor types.

The described upstream process is part of a larger study, describing development and optimization of an adenovirus production process, ranging from upstream virus production to a purified sterile-filtrated bulk product. Although adenovirus production was subject for this study, other types of viruses could well be produced using the described process, including virus propagation in HEK293 cells cultured in CDM4HEK293 medium using the ReadyToProcess WAVE 25 system.

References

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3. Application note: Evaluation of HEK293 cell growth and adenovirus productivity in HyClone CDM4HEK293 medium. GE Healthcare, 29264715, Edition AA (2017).
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Ordering information

Product	Description	Product code
CDM4HEK293	1 L	SH30858.02
L-glutamine 200mM	100 mL	SH30034.01
ReadyToProcess WAVE 25	Rocker	28988000
ReadyToProcess CBCU Full	Control unit	29044081
Cellbag Bioclear 11	Cellbag, 10L, BC11, pHOPT, DOOPT II	CB020L11-31
Cellbag Fortem	Cellbag, 10L, BC11, pHOPT, DOOPT II	CB0020L722-31
IN Cell Analyzer 2200	Cell imaging system	29027886

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