Adenovirus production in single-use Xcellerex XDR-10 bioreactor system

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Adenovirus production in single-use Xcellerex™ XDR-10 bioreactor system

This application note describes the production of adenovirus in HEK293 cells cultured in HyClone™ CDM4HEK293 culture medium. Early process development was performed in shake flask cultures, aiming for a suitable process for scale-up to single-use bioreactor culture. Comparable results were obtained in triplicate runs performed using the XDR-10 bioreactor system, with similar culture performance as in shake flask control cultures. The results indicate a robust and scalable adenovirus production process.

Introduction

Adenovirus-based vectors have been widely evaluated as vaccine delivery system in preclinical and clinical studies of various infectious diseases (1). Adenovirus has also been extensively 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.

Manufacturing of safe and efficacious clinical-grade viruses relies on a scalable and cost-effective production process. Early-stage studies are often performed using anchorage-dependent cells cultured in roller bottles or cell factories. However, scale-up using these techniques is complicated and limited by the surface area available for the cells to grow on. One attractive alternative for easier scale-up of adherent cell cultures is the use of microcarriers.

Another solution is to use a suspension-adapted cell line, which more easily lends itself to scaling. HEK293 suspension cell growth and adenovirus productivity in CDM4HEK293 medium has been previously evaluated (3). This work aims to demonstrate scale-up from shake flask to bioreactor cultures, with consistency in adenovirus production in CDM4HEK293 medium using the XDR-10 bioreactor system in triplicate runs.

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
Filling volume	3.5 L
Inoculum volume	Approx. 600 mL
Operating volume	4–10 L
Agitation/Impeller speed	100 rpm, down pumping
Temperature	37°C
pH set-point	7.1 (controlled by CO_2 and $NaHCO_3$)
pH PID settings	Factory default settings
Base for pH control	7.5% (w/v) $NaHCO_3$
DO set-point	40% (controlled by oxygen enriched air)
DO PID settings	Factory default settings
Spargers	0.5 mm: air, CO_2 20 µm: air, O_2
Oxygen flow	PID controlled
Carbon dioxide flow	PID controlled
Antifoam	10% Antifoam C (when needed)

Materials and methods

Bioreactor cultures

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 in 3.5 L prewarmed CDM4HEK293 medium in a 10 L XDA cell culture Pro bag. Culture working volume at start was between 4 and 4.5 L. When viable cell density (VCD) reached $1.6–2.0 \times 10^6$ cells/mL, complete CDM4HEK293 medium was added to the culture to reach a VCD slightly below 1×10^6 cells/mL to allow for addition of nutrients and dilute metabolites before infection. Final working volume was 8–10 L. The process was performed in three consecutive runs, using culture parameters listed in Table 1.

Shake flask control cultures

HEK293.2sus cells were expanded in complete CDM4HEK293 cell culture medium in shake flasks. Control cultures were run in duplicate for each bioreactor culture, using culture parameters listed in Table 2.

Table 2. Parameters used for shake flask cultures

Production medium	CDM4HEK293 supplemented with 4 mM L-glutamine
Starting viable cell density	0.3×10^6 viable cells/mL
Starting volume	100-130 mL in a 500 mL shake flask
Volume at infection	200 mL
Agitation	100 rpm
Temperature	37°C
CO_2	5%
Humidity	80%

Virus propagation

Cell cultures were infected with E1/E3-deleted AdV5, coding for green fluorescent protein (GFP), at a time of infection (TOI) of 1×10^6 cells/mL and a multiplicity of infection (MOI) of 10. Time of harvest (TOH) was 42 h post infection, and virus was released by detergent treatment.

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™ analyzer (Beckman Coulter). Glucose, lactate, ammonium, glutamine, and glutamate 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.

Percentage of infected cells was analyzed by flow cytometry at 24 h post infection using BD Accuri™ C6 (BD Biosciences). Non-infected HEK293.2sus cells were used as negative control.

Infectious virus titer in harvest samples was analyzed using adherent HEK293 cells. A constant number of cells were infected with serial dilution of the sample, and GFP-infected cells were detected and counted after 42 h by automated fluorescence microscopy using the IN Cell Analyser 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.

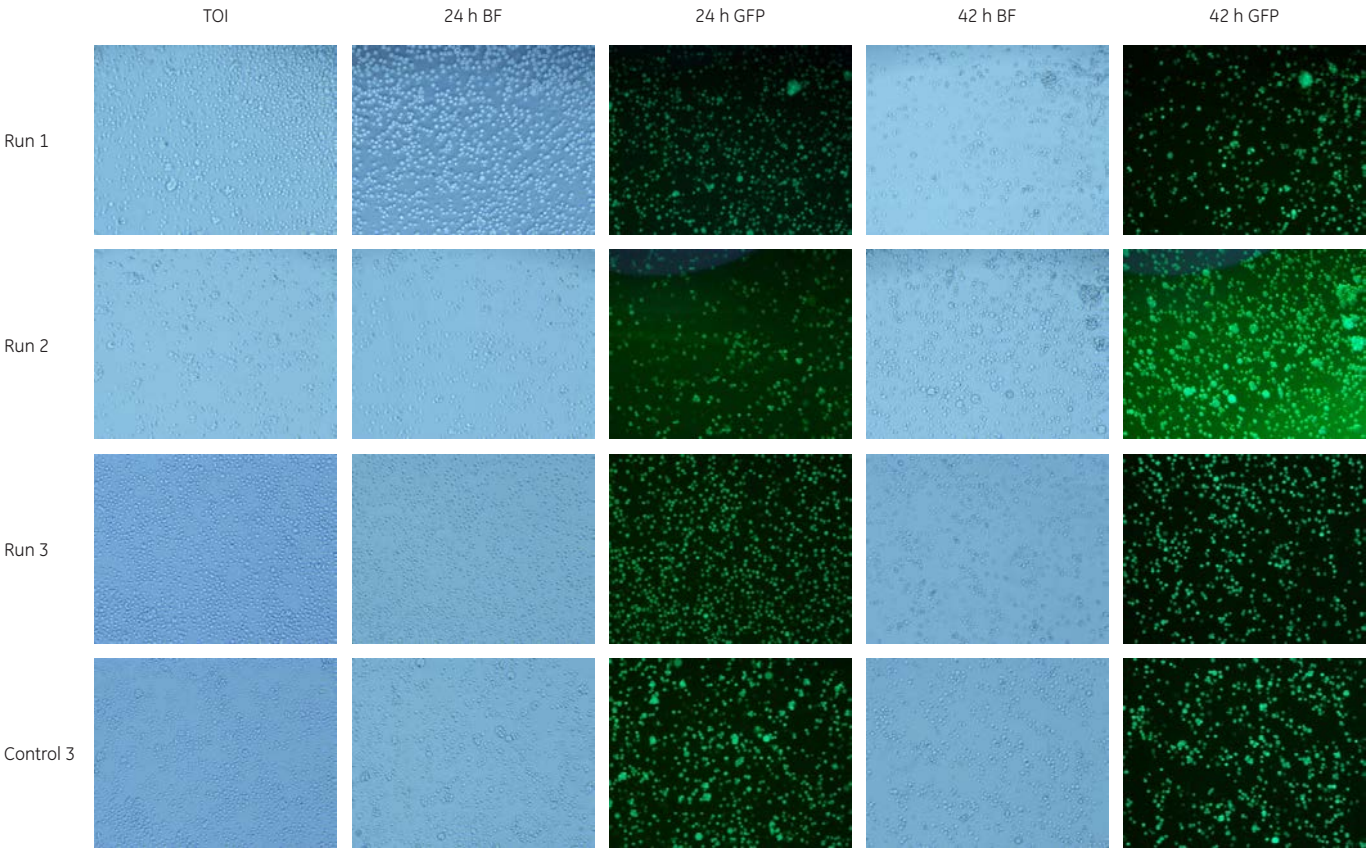

Fig 1. Cell morphology over the culture period. TOI = time of infection, BF = bright field, GFP = green fluorescent protein.

Results

Cell morphology and aggregates were monitored daily and the results from TOI until harvest are shown in Figure 1. Culture samples were taken daily for analysis of VCD and viability (Fig 2). Glucose, glutamine, glutamate, lactate, and ammonium (NH_3) were determined from time of inoculation (day 0) until time of infection (day 3) (Fig 3). Cell growth and viability as well as nutrient and metabolite levels were similar between runs. In bioreactor culture Run 1, culture pH was monitored daily both online and offline. For bioreactor culture Runs 2 and 3, culture pH was monitored daily online, while offline pH was monitored until time of infection. Results from the pH measurements presented in Figure 4 show the tight pH control in the bioreactor cultures compared with the uncontrolled pH in the shake flask cultures.

Amount of infected cells at 24 h post infection was between 92% and 96%. Virus titer was measured with two methods: automated fluorescence microscopy (IN Cell) and qPCR. Both methods show consistent results between the bioreactor and the shake flask control cultures (Fig 5). Based on our analytical methods, the ratio total (qPCR) to infectious (IN Cell) virus particles is as what can be expected.

Three comparable runs using the XDR-10 bioreactor system was successfully completed. The results indicate a robust production process for adenovirus in HEK293 suspension cells.

Similar results were obtained in the bioreactor runs as in the shake flask control cultures, indicating good scalability between the culture formats. An advantage of performing the process in a bioreactor system compared with in shake flasks is the scale at which the process can be performed. The Xcellerex XDR bioreactor family enables scaling of the process up to 2000 L. In addition, the bioreactor system allows pH and DO to be tightly controlled within the ranges of choice.

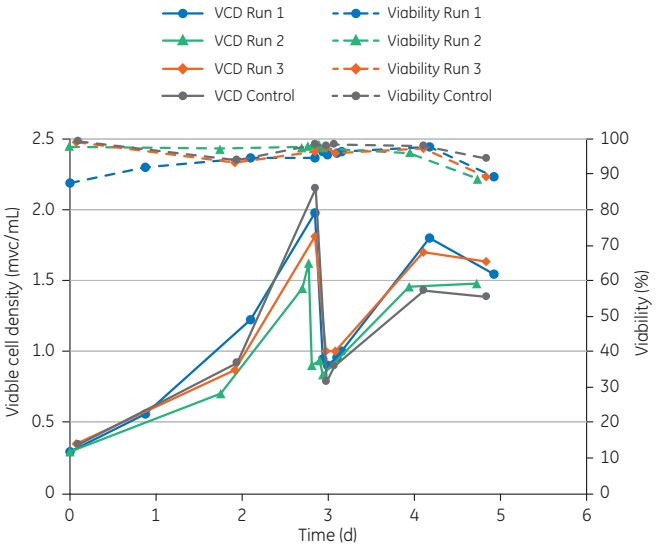

Fig 2. Viable cell density and viability in XDR-10 bioreactor Run 1–3 and in shake flask control culture Run 3. After addition of cell culture medium on day 3, cells were infected when reaching 1 × 10^6 cells/mL.

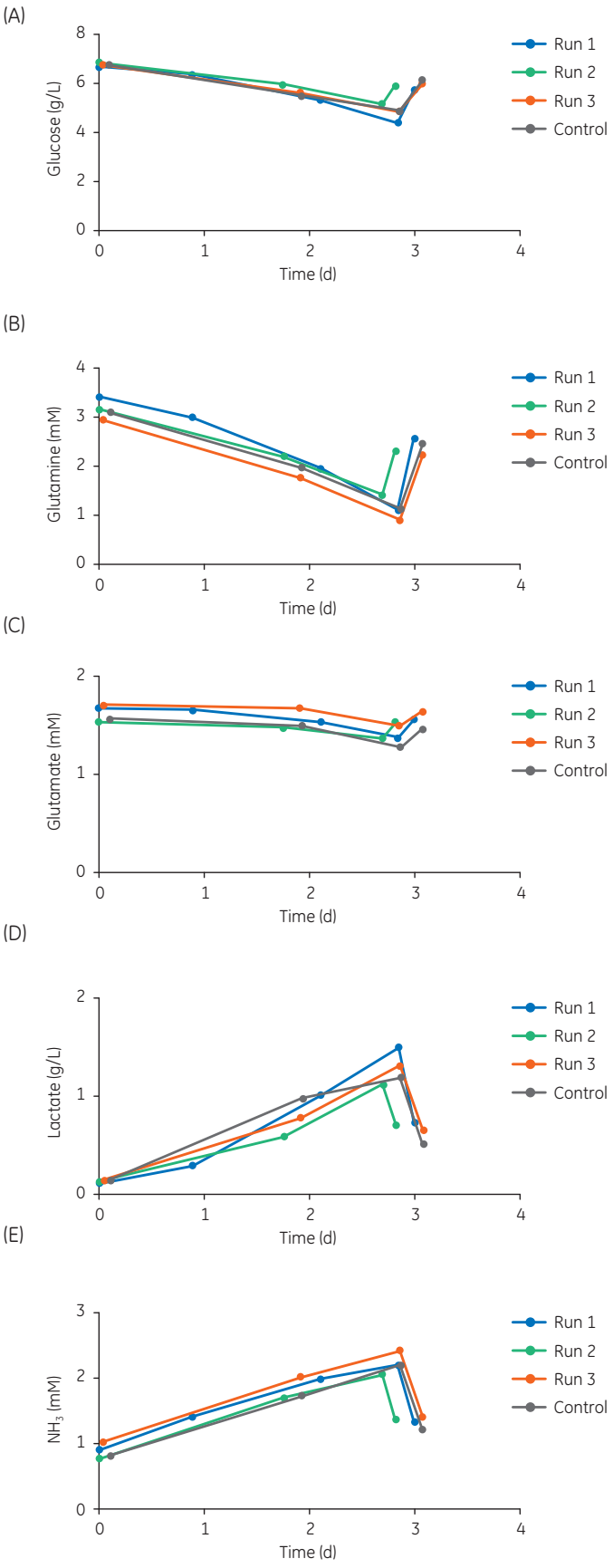

Fig 3. Culture concentrations of (A) glucose, (B) glutamine, (C) glutamate, (D) lactate, and (E) ammonium (NH_3) in XDR-10 Run 1-3 and in shake flask control culture Run 3.

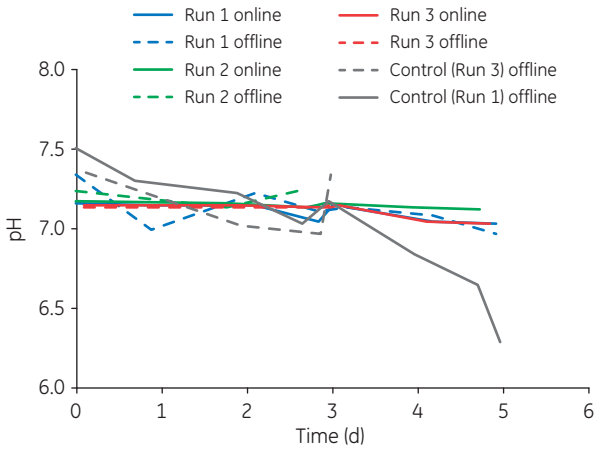

Fig 4. Culture pH measured both offline (bioreactor and shake flasks) and online (bioreactor).

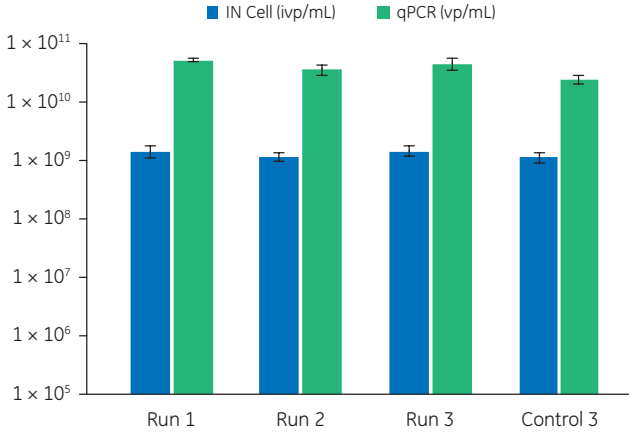

Fig 5. Virus titers determined using IN Cell and qPCR. Results are based on a minimum triplicate analyses.

Conclusion

This work demonstrates production of adenovirus in HEK293 cells cultured in CDM4HEK293 medium using the XDR–10 bioreactor system. Similar cell growth and adenovirus productivity was achieved in three comparable runs, indicating process robustness. Similar results were also obtained when the culture process was performed in shake flasks, showing good scalability of the process. The described upstream process is part of a project for development of an adenovirus production process, ranging from upstream virus production to a purified sterile-filtrated bulk product.

References

1. Kallel, H. and Kamen, A.A. Large-scale adenovirus and poxvirus-vectored vaccine manufacturing to enable clinical trials. *Biotechnol J* **10**, 741–747 (2015).
2. Appaiahgari, M.B. and Vrati, S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. *Expert Opin Biol Ther* **15**, 337-351 (2015).
3. Application note: Evaluation of HEK293 cell growth and adenovirus productivity in HyClone CDM4HEK293 medium. GE Healthcare 29264715, Edition AA (2017).

Ordering information

Product	Description	Product code
CDM4HEK293 medium	1 L	SH30858.02
XDA-10 Pro bioreactor bag	10 L, pitch blade impeller, one disc each of 2 µm, 20 µm, 0.5 mm, and 1 mm spargers.	888-2-0396-C
Probe Sheath	An accessory to the disposable bag that provides asceptic connection of a probe with the cell culture inside the bag.	888-0138
L-glutamine 200 mM	100 mL	SH30034.01
IN Cell Analyzer 2200	Cell imaging system	29027886

To order the XDR-10 system, please contact your local sales representative.

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