Evaluation of HEK293 cell growth and adenovirus productivity in HyClone CDM4HEK293 medium

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Human embryonic kidney 293 (HEK293) cells are widely used in production of recombinant viruses and proteins because of their stable growth, high transfection level, and ability to produce high viral titers. HEK293 cells are susceptible to infection by adenovirus, one of the most frequently used viral vectors in the development of therapeutic vaccines and vaccines against infectious diseases. This application note demonstrates growth of HEK293 cells in CDM4HEK293 medium in both shake flask and bioreactor cultures. Adenovirus productivity was evaluated in shake flask cultures. Performance in CDM4HEK293 was compared with performance in a reference medium.

Introduction

Adenovirus-based vectors have been widely evaluated as vaccine delivery system in preclinical and clinical studies for infectious diseases such as rabies, influenza, and human immunodeficiency virus (1). Several generations of recombinant adenovirus vectors have been developed to enhance safety and productivity and to produce more effective vaccines (2). However, the most studied adenovirus vector is the first generation of adenovirus 5 (AdV5), making this a suitable model for process development of recombinant adenoviral vectors.

The HEK293.2sus cell line supports replication of E1/E3-deleted recombinant AdV5. Optimal cell performance, however, depends on several factors such as growth and productivity of the specific cell clone, composition of the culture medium, and culture conditions such as agitation, aeration, and temperature. An understanding of how these factors influence metabolic processes is essential when designing cell culture media. Knowledge about a cell line's metabolic activity, nutritional requirement, and waste creation helps to ensure that the correct combination and amounts of nutrients are used to minimize waste generation, which can result in cell toxicity.

CDM4HEK293 is a protein-free and animal-derived component-free medium that promotes adenovirus and recombinant protein production. The medium was developed to support high viable cell density and productivity of HEK293 cells in suspension cultures. Cell growth, viability, and adenovirus productivity of HEK293.2sus cells cultured in CDM4HEK293 was compared with performance of the same cells in the reference medium in shake flask cultures. Scalability of the process in CDM4HEK293 medium was investigated in Xcellerex™ XDR-10 bioreactor and the performance was compared with the shake flask cultures.

Materials and methods

Cell medium adaptation

HEK293.2sus cells (ATCC) were adapted to two different serum-free media: HyClone CDM4HEK293 medium and Gibco™ CD293 (Thermo Fisher Scientific) medium (reference). Cell adaptation was performed by transferring cells grown in Gibco CD293 medium into new medium sequentially (100%/0%; 75%/25%; 50%/50%; 25%/75%; 12.5%/87.5%; 0%/100%), using a seed cell density of 5.0×10^5 cells/mL at each step.

The cells were subcultivated every three to four days or when two population doublings had been reached. Subculturing of the cells were conducted by mixing cell suspension in conditioned medium and new medium. Each step in the sequential adaptation included two to three subcultivations using the same medium ratio. Subcultivation of the cells using this method continued until the initial medium was reduced to 0% (with a cell viability above 90%).

Shake flask cultures

HEK293.2sus cells were seeded into 30 to 50 mL of either CDM4HEK293 or reference medium supplemented with 4 mM L-glutamine at a cell density of 0.4×10^6 cells/mL in 125 mL shake flasks. Culturing was conducted in a shaker incubator at 100 rpm, 37°C, and 5% CO_2. Cultures were terminated when cell count and viability started to decline. Culturing was performed in triplicate runs.

Virus propagation

Cell cultures were infected with E1/E3-deleted AdV5, coding for green fluorescent protein (GFP), at a predefined time of infection (TOI) of 1 × 10^6 cells/mL and a multiplicity of infection (MOI) of 1 or 10. Time of harvest (TOH) was performed between 42 to 48 h post infection, and virus was released by three cycles of freezing and thawing.

Infectious virus titer

Adherent HEK293 cells were grown in 96-well microplates and infected with 10-fold serial dilutions of crude virus lysate. After 48 h incubation, infected cells expressing GFP were detected by automated flouresence microscopy using the IN Cell Analyser 2200.

Bioreactor cultures

HEK293.2sus cells were seeded at a cell density of 0.25 × 10^6 cells/mL into 1 L of supplemented CDM4HEK293 medium. The seed culture was transferred to single-use XDA 10 L cell culture Pro bag in a total final bioreactor batch volume of 8 L. Cells were cultured in the XDR-10 bioreactor system using culture parameters listed in Table 1.

Table 1. Parameters used for bioreactor cultures

Culture medium	CDM4HEK293 + 4 mM L-glutamine
Working volume	8 L
Impeller speed	100 rpm (down-flow direction)
Temperature	37°C
pH set point	7.1 (controlled by CO_2 and base)
DO set point	40% (controlled by oxygen enriched air)
O_2 flow	PID controlled
CO_2 flow	PID controlled

Culture analysis

The cultures were sampled over the culture period for analysis of cell growth, viability, morphology, metabolites, nutrient consumption, and osmolality. Cell growth and viability were determined using the Vi-CELL™ analyzer (Beckman Coulter). Glucose (GLC), lactate (LAC), ammonium (NH_3), glutamine (GLN), and glutamate (GLU) were measured using the CEDEX™ Bio analyser (Roche). Cell morphology in shake flask cultures was examined microscopically over the culture period. Osmolality in bioreactor cultures was determined using the Bioprofile FLEX™ analyzer (Nova Biomedical Corp.).

Results

Shake flask cultures

HEK293 cells were cultured in either CDM4HEK293 or in reference medium for two weeks or until viability dropped. Samples were taken daily to measure growth and viability of the cell cultures (Fig 1). For the CDM4HEK293 medium, a cell count of more than 5.5 × 10^6 cells/mL with good viability was reached, while for the reference medium, a cell count of approximately 3.5 × 10^6 cells/mL was achieved.

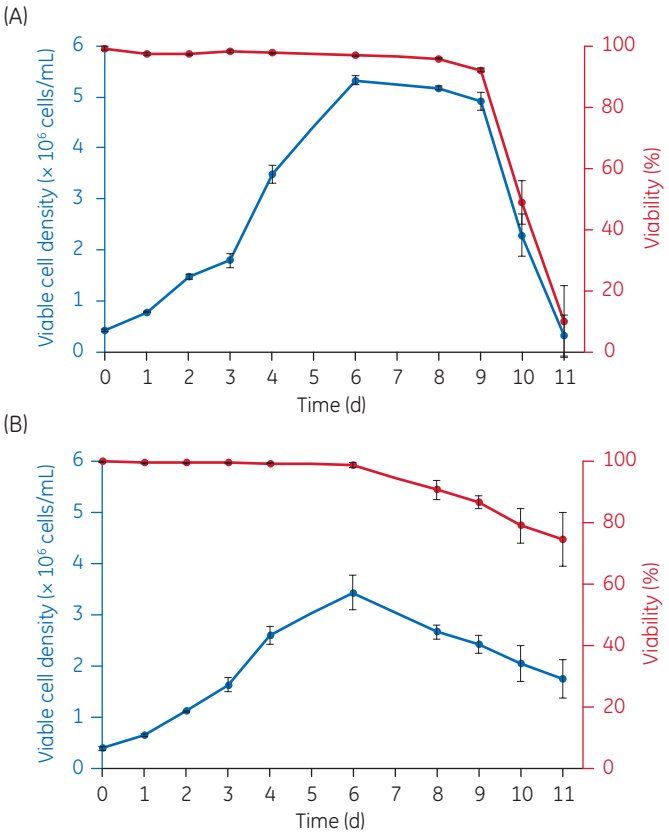

Fig 1. Cell growth and viability in (A) CDM4HEK293 medium and (B) reference medium for triplicate shake flask batch cultures.

The cultures were sampled daily or every second day for analysis of cell nutrients and metabolites (Fig 2). An initial glucose concentration of close to 7 g/L in the CDM4HEK293 cultures could partly explain the high viability of the cells over the culture period. The rapid initial decrease in glutamine levels could explain the initiation of increasing ammonia in the cultures. Glutamate concentrations were found to be slightly increased in the CDM4HEK293 cultures, whereas lactate levels were similar between the media.

Cell morphology and aggregation was examined microscopically over the culture period, and representative images from 24 and 96 h after inoculation are shown in Figure 3.

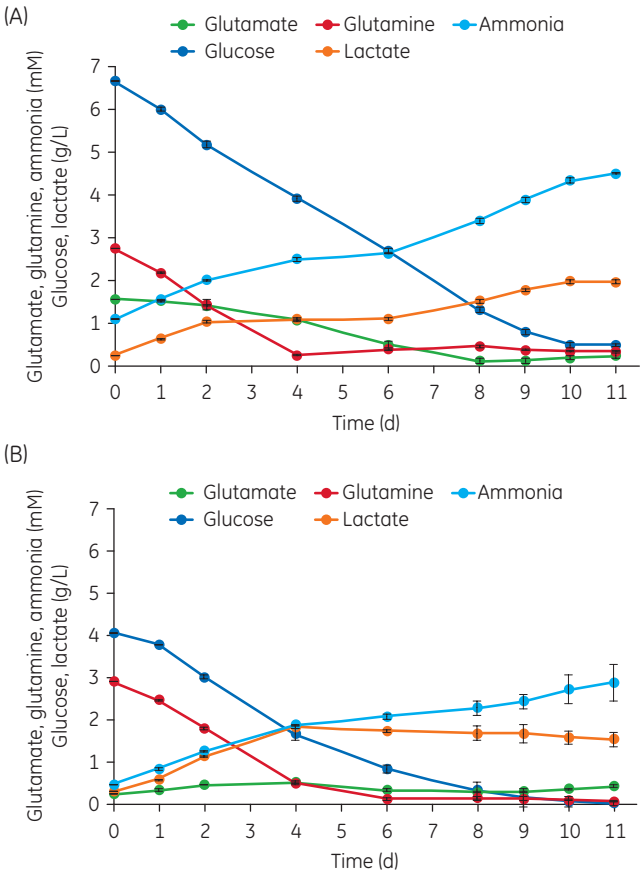

Fig 2. Nutrient and metabolite concentrations in (A) CDM4HEK293 medium and (B) reference medium in triplicate shake flask batch cultures.

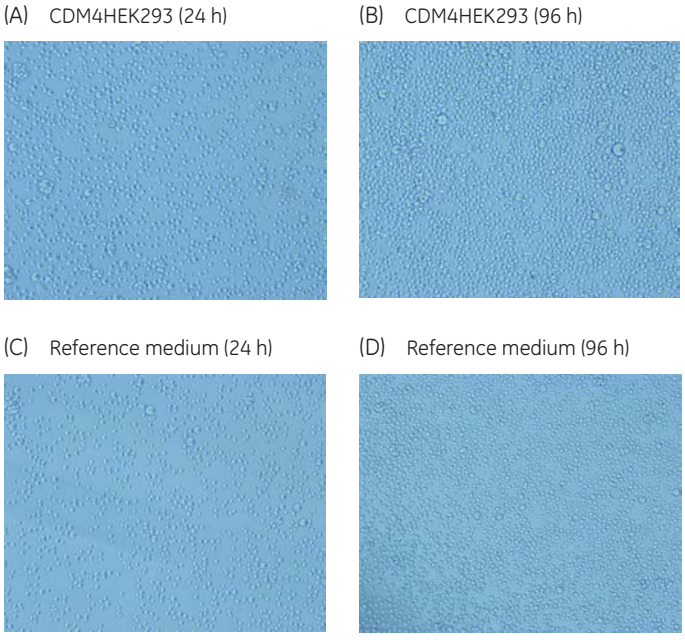

Fig 3. Cell morphology at 24 and 96 h after inoculation in the (A, B) CDM4HEK293 medium and (C, D) reference medium in shake flask batch cultures.

Cells cultured in CDM4HEK293 and infected with AdV5-GFP show a higher infectious titer than cells cultured in reference medium (Fig 4).

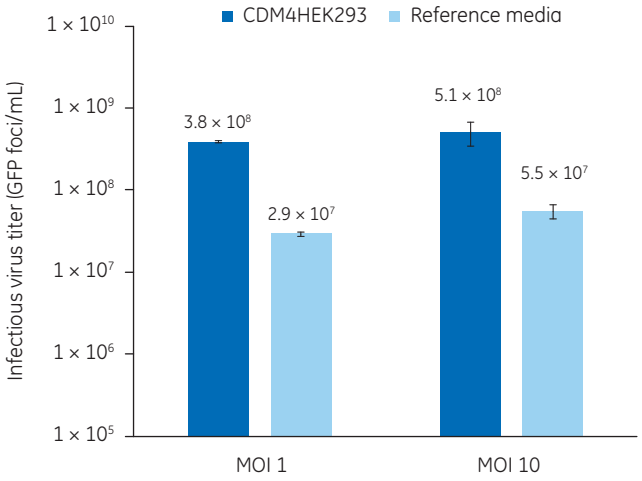

Fig 4. Infectious virus productivity in shake flask cultures using CDM4HEK293 or reference cell culture medium.

Bioreactor cultures

To demonstrate scalability of the CDM4HEK293 cultures, HEK293 cells were cultured using the XDR-10 bioreactor system. Cells were cultured in an 8 L final working volume until cell growth and viability started to decrease. Peak cell density and viability were about 5 × 10^6 cells/mL and 95%, respectively (Fig 5). The results are similar to what was achieved in the shake flask cultures.

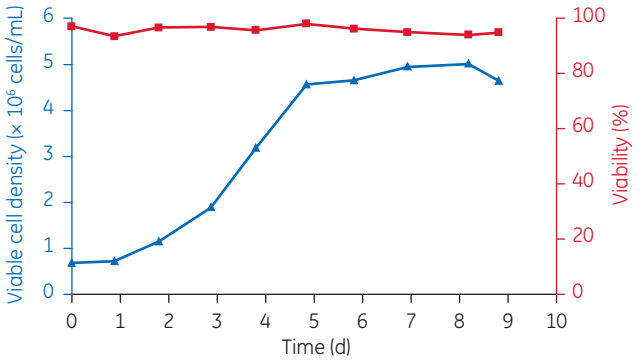

Fig 5. Cell growth and viability in 8 L CDM4HEK293 bioreactor culture.

Nutrients and metabolites in bioreactor cultures (Fig 6) exhibit similar profiles as in shake flask cultures (Fig 2A). As nutrients were consumed, metabolites accumulated. At day 8 post inoculum at peak cell count, nutrients were low and metabolite concentrations had increased significantly since culture initiation.

A pH set point of 7.1 was used, and pH was monitored daily in the bioreactor cultures. The pH was maintained stable at set point and the osmolality was maintained stable within the range of 300–325 mOsm/kg over the culture period (baseline osmolality was 307 mOsm/kg).

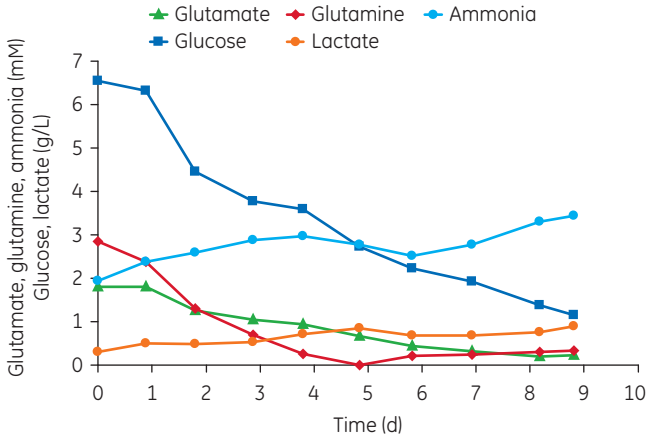

Fig 6. Nutrient and metabolite concentrations in 8 L CDM4HEK293 bioreactor culture.

Conclusion

This application note demonstrates growth and adenovirus productivity of HEK293 cells grown in shake flasks in CDM4HEK293 medium or in a reference medium. The results show an improved cell growth and infectious virus productivity in CDM4HEK293 medium as compared with in the reference medium. To demonstrate scalability of the CDM4HEK293 cultures, performance of cultures grown in a XDR-10 bioreactor system was compared with the shake flask cultures. The results show a similar performance between the culture systems, confirming the suitability of using CDM4HEK293 as a platform medium for HEK293 cell culture applications.

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).

Ordering information

Product	Description	Product code
XDR-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. Packsize: 4	888-0138
HyClone CDM4HEK293 medium	1 L	SH30858.02
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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