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Platform for the Growth and Propagation of HEK293 Cells and Adenovirus Viral Vector Amplification

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BACKGROUND

Industrialization of viral vector production for gene therapy applications demands consistent processes utilizing robust production platforms and raw materials that can meet quality standards and regulatory compliance. Typical vector production schemes involve propagating seed trains and viral amplification in cultures utilizing serum containing media. Although serum-containing media systems deliver high viral vector yields, the serum component has certain disadvantages such as performance variability, high costs, and potential of introducing adventitious agents. Utilizing serum-free chemically defined medium for growth and amplification addresses these concerns. Here we describe a highly efficient and economical single-use bioreactor platform for producing gene therapy viral vectors with flexibility of using serum-containing or serum-free media.

We evaluated multiple serum-free chemically defined media formulations for adenovirus vector production in the single-use fixed-bed iCELLis® bioreactor. These media were evaluated for ease of adaptation and growth characteristics with HEK293 cells previously adapted to serum-free growth conditions. Process parameters such as multiplicity of infection (MOI) and duration of infection (DOI) were optimized in a shake flask to maximize µvector amplification.

The process was then transferred into the iCELLis Nano bioreactor. The HEK293 cells were maintained under adherent conditions and growth was monitored until the cell densities reached approximately 200,000 cells/cm^2. Complete medium exchange was performed in iCELLis Nano bioreactor without cell removal, followed by viral infection at MOI 50. Harvest was performed 72 hours post infection.

Viral titers were quantitated using an adenovirus in-vitro infectivity assay. Viral productivity was generally consistent between the shake flask and the iCELLis Nano bioreactor system and between serum-containing or serum-free chemically defined medium. These studies demonstrate the iCELLis bioreactor as a versatile scalable technology, and support its use for production of gene therapy vectors in processes using serum-containing or serum-free media.

MATERIALS AND METHODS

Growth and Adaptation

- 2 HEK293 chemically defined (CD) media formulations, benchmarked against a 3rd CD control media
- Suspension cells, 293-H (Thermo Fisher Scientific)
- 125 mL shake flask cultures, appropriate CO_2, maintained 3x10^5 – 2x10^6 cells/mL
- Cell densities were measured by Vi-CEL♦ (Beckman Coulter) using Accumax♦ (Innovative Cell Technologies)

Ad5 Amplification Optimization

- Cultures grown to 2.0 x 10^6 cells/mL. Complete media exchange, infection at MOI of 10, 50 and 100
- Samples collected at 48 and 72 hours post infection
- Infectivity measured, spent media and cell lysis fractions Adeno-X♦ Rapid Titer Kit (Takara)

Production in iCELLis Nano Bioreactor

- CD media benchmarked against serum-containing media (DMEM with 10% fetal bovine serum [FBS])
- Bioreactor grown to 200,000 cells/cm^2
  Complete media exchange, infection at MOI 50
  Harvest 72 h post infection
- Infectivity measured, spent media and cell lysis fractions; Adeno-X Rapid Titer Kit

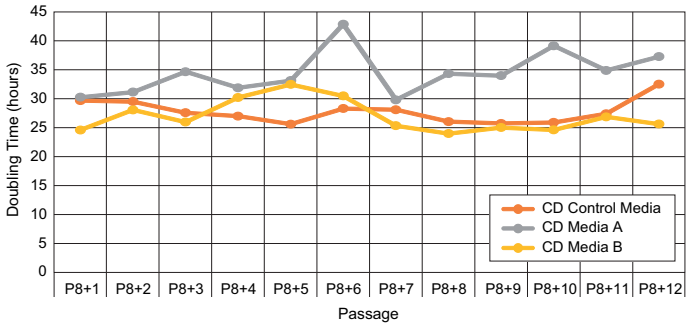
RESULTS AND DISCUSSION

Growth and Adaptation to Chemically Defined Media

293-H cells were directly adapted to three formulations of CD media, each from a different vendor. Growth was monitored for 12 passages in shake flask. Doubling times calculated, shown in Figure 1.

Figure 1

Growth and adaption of 293-H cells in chemically defined media



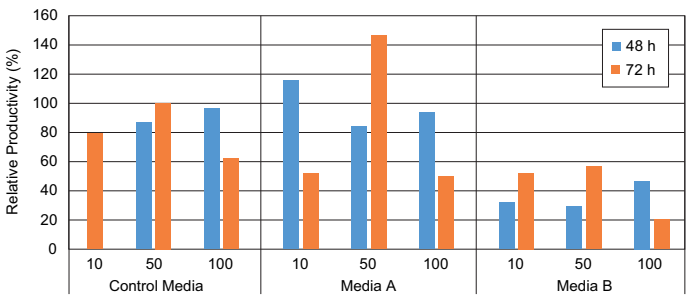
- Average doubling time was similar between the control media and media B; media A was slower than the control media
- The average doubling times are between 27 and 34 hours, slow but acceptable

Optimization of Adenovirus Amplification

Process parameters including MOI and DOI were optimized in 125 mL shake flasks. Total vector productivity was normalized to the MOI 50, 72-hour control media. This data is shown in Figure 2.

Figure 2

MOI and DOI optimization to maximize adenovirus productivity



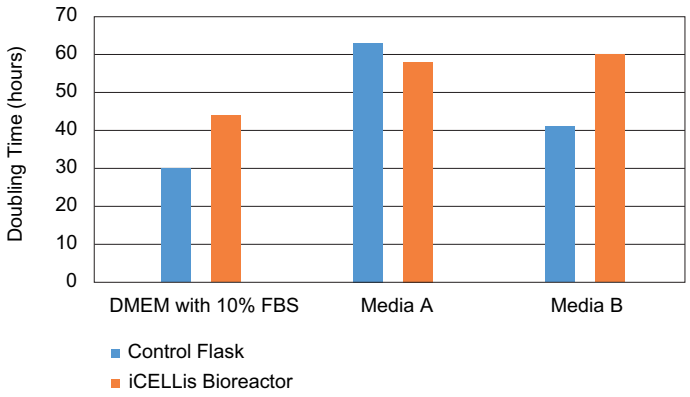
- Highest productivity for all 3 media formulations was achieved with an MOI of 50 and 72-hour DOI
- Media A resulted in a ~40% increase in productivity over the control media, whereas media B produced only 60% of the control media

Chemically Defined Media Performance in the iCELLis Bioreactor

Media A and media B were then used to scale production to the iCELLis Nano bioreactor. These media were benchmarked against standard serum containing media (DMEM + 10% FBS).

Figure 3

Comparison of growth rate in the iCELLis bioreactor and control flask

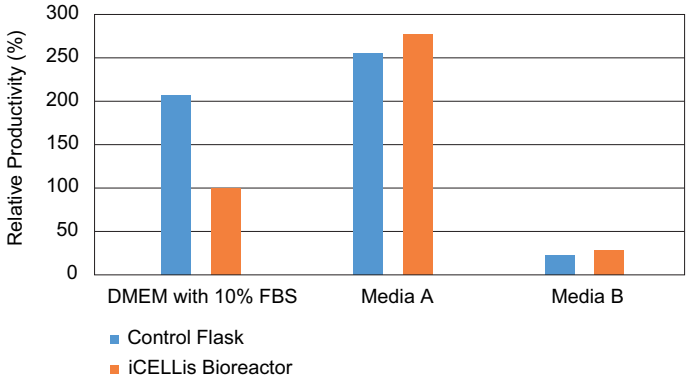


- The growth rate in the iCELLis bioreactor is slower than the control flask for media B and the DMEM with 10% FBS control media
- Metabolic data showed higher glucose consumption in the control flasks compared to iCELLis bioreactor (data not shown)
- No significant amounts of cells were found in the recirculating media indicating >95% of the biomass was contained in the packed bed (data not shown)

The bioreactor was infected with Ad5 as described in the methods section. Total productivities were normalized to the productivity in the serum-containing control media in the iCELLis bioreactor. Results are shown in Figure 4.

Figure 4

Ad5 productivity in the iCELLis bioreactor with chemically defined media



CONCLUSION

- Overall productivity in media A was significantly higher than the productivity with both media B and the DMEM with 10% FBS control media
- Productivity was generally consistent in the iCELLis bioreactor and the control flasks for the two chemically defined media formulations. Productivity was lower in the iCELLis bioreactor with control media compared to the control flask
- iCELLis bioreactor can be used to produce adenovirus with serum-free, chemically defined media with viral productivities similar to or greater than serum-containing media

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