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Application of Aber Biomass Probes to Inform Transfection Timing in Chemically Defined iCELLis® Bioreactor-Based Viral Vector Manufacturing

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INTRODUCTION

Objective

- To demonstrate that the Aber Futura♦ biomass probe can be used to accurately determine optimal transfection cell density, as cell density at the time of transfection (TF) is a major determinant of overall vector productivity:
  - Over/under confluent cultures can yield poor productivity
  - Nuclei-based counting is time consuming and reproducibility is limited
- To demonstrate practical application of biomass probes to maximize vector production and reproducibility in Pall's iCELLis bioreactor

iCELLis Bioreactor Background

- Provides clinical and commercial manufacturing of viral vectors and vaccines from adherent cells by utilizing:
  - Cell substrate composed of medical grade, uncoated, uncharged polyethylene teraphalate (PET) carriers
  - A closed system with reduced footprint and minimal aseptic handling
- Controls pH and dissolved oxygen (DO) through a falling film that provides:
  - High gas transfer rates due to large surface area and thin film mass transfer, no sparging required
  - No cell shear due to rising bubbles or bubble burst
- Exhibits bench- and commercial-size scalability by maintaining fixed bed height and carrier compaction

Pall's iCELLis 500+ bioreactor

Aber Futura Biomass Probe Background

- Provides continuous, online, direct measurements of biomass by inducing polarization of viable cells and measuring the resulting capacitance in pF/cm
- Allows measurements of cell biomass during a viral vector production run through integration into the iCELLis bioreactor
- Provides invaluable real-time information regarding cell growth and health, elucidating optimal times for operations such as:
  - Control of feed/perfusion rate
  - Transfection
  - Harvest

InVitria OptiPEAK♦ HEK293t Serum-Free Media (SFM) Background

- Chemically defined media that is optimized specifically for adherent HEK-based adeno-associated virus (AAV) and lentivirus (LV) vector production in the iCELLis bioreactor:
  - Reduces foaming and other technical challenges in bioreactors
  - Provides high performance in culture, both for cell expansion and viral titer
- Developed to circumvent the high variability and cost found in classical serum supplemented media for virus production:
  - Creates more consistent cell growth and more dependable manufacturing yield by reducing donor-dependent variation
  - Eliminates concerns about the global supply chain and limitations on availability of human and bovine serum
- Utilizes a recombinant human transferrin and albumin from a large-scale non-mammalian expression platform and therefore does not incorporate any human or blood-derived components:
  - Prevents the introduction of undesirable viral RNA and DNA, prion proteins, undefined antigenic molecules, and human or animal cytokines and hormones
  - Uses blood-free components to eliminate adventitious pathogenic agents that can be found in blood-derived products

METHODS

- HEK293 (AAV) or HEK293T (LV) cells were expanded in flatware either in OptiPEAK HEK293t media or DMEM+10% fetal bovine serum (FBS) as seed stock for 5,300 cm² iCELLis Nano bioreactors. Cells were media-adapted for at least three passages prior to seeding in the bioreactors
- Key bioreactor parameters are described in Table 1
- Capacitance readings from the biomass probes were converted to cells/cm² as determined by the formula: *Nuclei count (cells/cm²) = m3 x capacitance* where m3 = 928.47 as described previously[1]
- PEI-mediated triple transfections of either AAV-2 GFP or LV GFP were performed at specific capacitance measurements
- Production times for each vector were 72 hours from transfection to harvest

Bioreactor Parameter	AAV Pre TF	AAV Post TF	Lentivirus Pre TF	Lentivirus Post TF
pH set point	7.30	7.30	7.00	6.80
Temperature set point (°C)	37	37	37	37
Linear speed (cm/s)	2	2	2	2
DO set point (%)	95	55	95	55

Table 1
iCELLis bioreactor set points for OptiPEAK HEK293t media

RESULTS

To determine optimal transfection densities in the iCELLis bioreactor with OptiPEAK HEK293t media, transfections using AAV and LV were done at different cell densities according to the biomass probes. Vector yield was plotted as a function of the highest titers achieved in this series of experiments (Figure 1). Transfections were performed anywhere between day two and day five post-inoculation of the bioreactor.

T-Flask Transfections with LV (HEK293T)

iCELLis bioreactor titer data suggested that lower transfection cell densities yielded higher vector titer for both AAV and LV. To correlate these observations made in the iCELLis bioreactor measured by the biomass probe to T-flasks measured by traditional cell counts, HEK293T cells were plated at different cell densities (Figure 2) in T-75 flasks and then transfected 16 hours later. Supernatants were analysed for functional LV titers in both DMEM+FBS and OptiPEAK HEK293t media. Optimal cell density at the time of transfection was determined to be approximately 75,000 cells/cm² for both media, aligning with previous iCELLis bioreactor results.

Collectively, the data suggested cell densities of 70,000 cells/cm² produced the highest titer of vector in the iCELLis bioreactor. Three serial runs of iCELLis Nano bioreactors using OptiPEAK HEK293t media were performed using the biomass probes (Figure 3). Bioreactors were transfected (arrow) when capacitance reached 74-80 pF/cm, corresponding to 68,000 to 74,000 cells/cm². Transfections were typically two days post inoculation. Total volume of virus-containing supernatant harvested from each bioreactor was 900 mL.

Average yield was determined to be $1.13 \times 10^7 \pm 0.27 \times 10^7$ IFU/mL. The coefficient of variation was found to be 24% between runs.

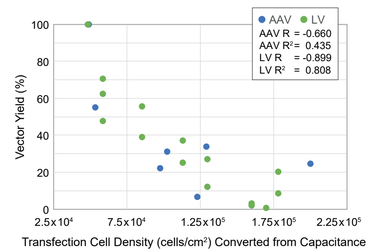


Figure 1
AAV (HEK293) and LV (HEK293T) titers at different transfection cell densities as determined by biomass probes

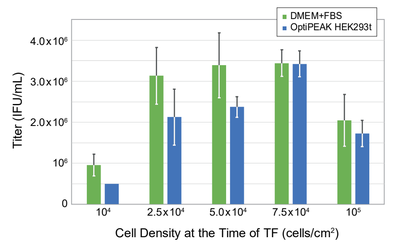
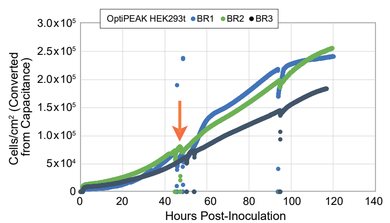


Figure 2
LV production in T-flasks



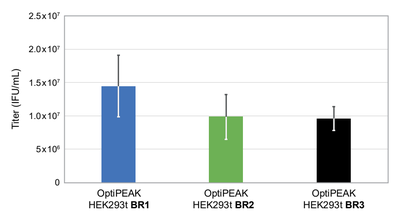


Figure 3
Independent iCELLis batches in OptiPEAK HEK293t media producing LV at optimized cell confluency. Biomass trends (left) and titer yield (right)

CONCLUSION

- **Biomass probes can accurately assess cell growth in the iCELLis bioreactor**
- **Optimal transfection cell density for AAV and LV production was determined to be approximately 70,000 cells/cm² in the iCELLis bioreactor using OptiPEAK HEK293t media**
- **Using capacitance to define the time of transfection led to high productivity of LV vector with acceptable reproducibility in chemically defined media**
- **Current work is focused on applying the same principle in iCELLis bioreactor-based AAV manufacturing**

Reference

1. Alfano, R. et al. (2020), Implementation of Aber's Futura Biomass Probe in Pall's iCELLis Nano Bioreactor Provides a Robust and Reproducible Method to Assess Cell Density [Poster], ASGCT, < https://www.pall.com/en/biotech/posters-presentations/reproducible-method-assess-cell-density.html>

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