

# Implementation of Aber's FUTURA<sup>®</sup> Biomass Probe in Pall's iCELLis<sup>®</sup> Nano Bioreactor Provides a Robust and Reproducible Method to Assess Cell Density

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## INTRODUCTION

### Objective

- Confirm Aber's FUTURA biomass probe provides reliable measurements when used with the iCELLis Nano bioreactor
- Demonstrate a relationship between cumulative glucose consumption, nuclei counts and capacitance
- Devise a theoretical method correlating iCELLis 500+ bioreactor capacitance to nuclei counts

### iCELLis Bioreactor Background

- The Pall iCELLis bioreactor has emerged as the leading workhorse for clinical manufacturing of viral vectors
- Single-use, fixed-bed bioreactor
- Bench- and commercial-size scalability by maintaining fixed-bed height and carrier compaction
- pH and dissolved oxygen (DO) control through gas exchange across a falling film of liquid media
- Compartmentalization – base addition, agitation, and fixed bed spatially separate
- Fixed bed acts as natural cell retention device for perfusion

### Aber's FUTURA Biomass Probe Background

- The biomass probe induces polarization of cells and measures the resulting capacitance of the medium in pF/cm, providing direct information on cell biomass
- The probe specifically detects viable cells with an intact cell membrane
- The capacitance method is insensitive to cell debris or viral particles
- Monitoring viable cell density with the biomass probe enables live online measurement of cell growth, ability to compare batch-to-batch to ensure reproducibility, control of feed/perfusion rate, identify optimal cell density for time of infection/transfection, and identify optimal time of harvest

### InVitria OptiPEAK HEK293t Background

- InVitria is a leader in chemically defined and clinic-ready cell culture media utilizing experience with viral vaccines, gene therapies and cell therapy defined media that delivers performance at least as robust as serum containing formulations without the problems associated with supply chain continuity, variability and regulatory challenges
- Combining OptiPEAK HEK293t medium and the iCELLis bioreactor can deliver enhanced performance, reliability, quality and consistency for clinical application in viral vaccines and gene therapies

## METHODS

- Three runs were performed using a 2.65 m<sup>2</sup> iCELLis Nano bioreactor
- Pre-adapted HEK293t cells were expanded and seeded using OptiPEAK HEK293t medium:
  - iCELLis 1 bioreactor was seeded at 8,000 cells/cm<sup>2</sup> while iCELLis 2 and 3 bioreactors were seeded at 26,000 cells/cm<sup>2</sup>
  - A media to surface area ratio of 0.187 mL/cm<sup>2</sup> was used for the growth phase
- iCELLis Nano bioreactor settings for OptiPEAK HEK293t medium were optimized based on growth characteristics from previous studies (Table 1)
- Time of inoculation is considered t = 0
- To demonstrate a relationship between nuclei counts, cumulative glucose consumption, and capacitance, correlations from 3 batches were used (Figure 2)
- Only pre-transfection data points, less than 300,000 cells/cm<sup>2</sup>, were included in correlations
  - Above 300,000 cells/cm<sup>2</sup>, cell size shrinks as cells pack into over confluency. The strength of the capacitance signal is affected by the cell size as well as total number of cells, so these data points would skew the correlation.

**Table 1**  
iCELLis Nano bioreactor settings

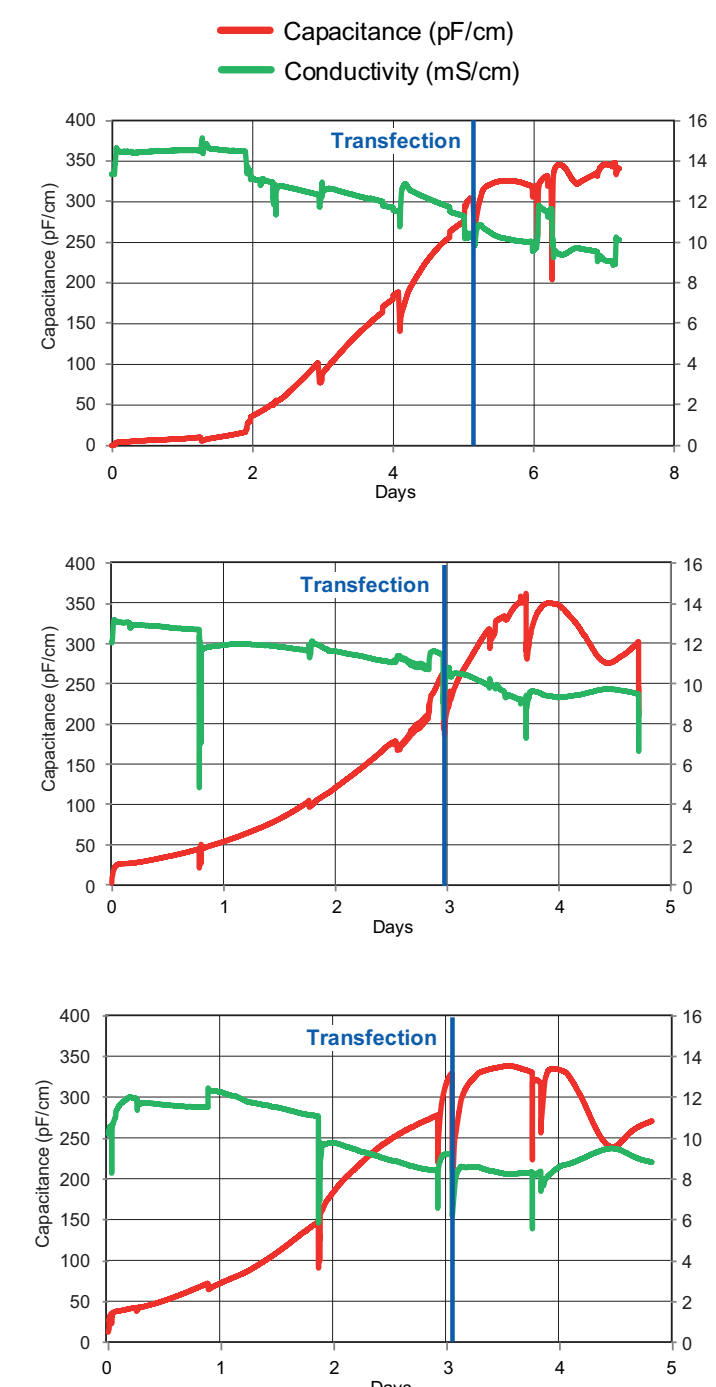
Media	pH Setpoint	Temperature Setpoint (°C)	Linear Speed Rate (cm/s)	DO (%)
OptiPEAK HEK293t medium	7.25	37	2	95



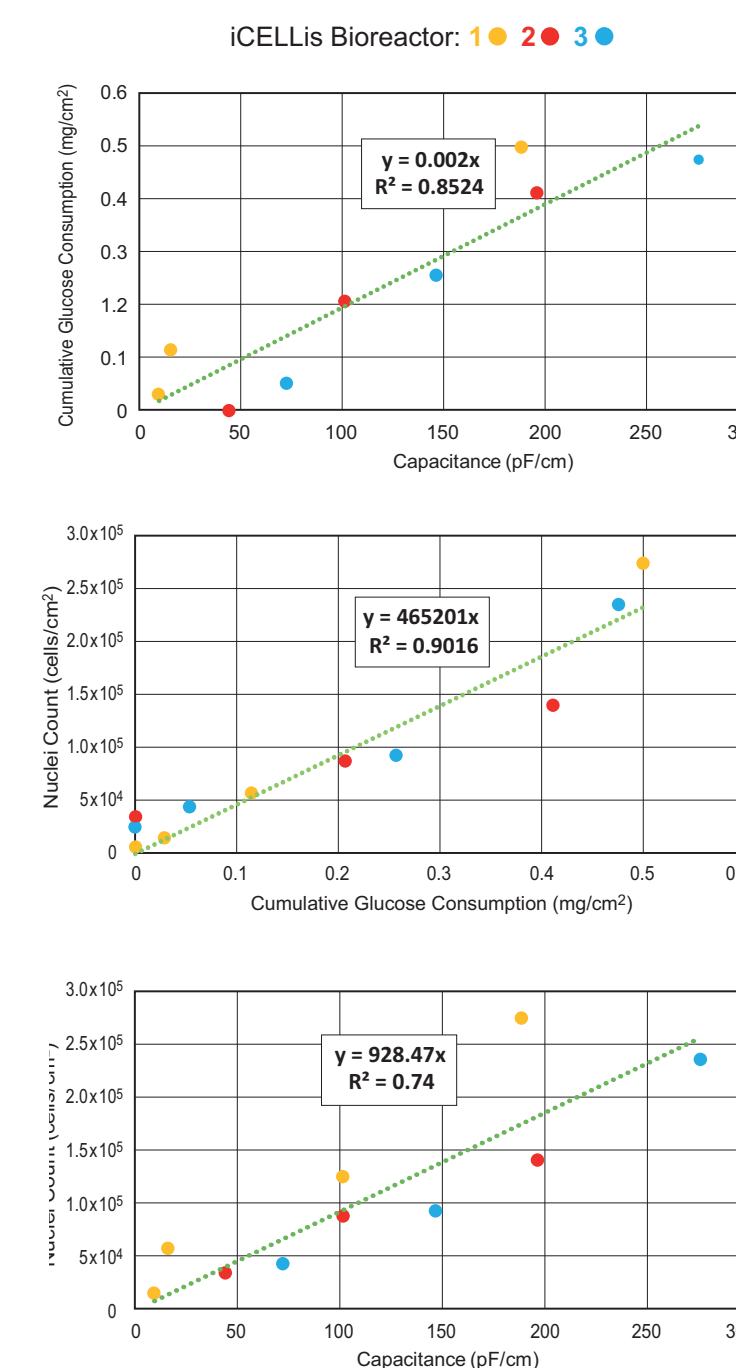
## RESULTS

- Aber's FUTURA biomass probe provided smooth, continuous, and reliable measurements of cell density throughout the run
- The capacitance measurement indicates that in all three cases, cells were transfected in the exponential growth phase

**Figure 1**  
Biomass probe trend lines of capacitance and conductivity for iCELLis 1, 2 and 3 bioreactors



**Figure 2**  
Correlations between nuclei counts, cumulative glucose consumption, and capacitance



**Note:** Higher R<sup>2</sup> values might be possible by using an established process and averaging of more biological runs

- From the linear regressions (Figure 2), the following equations can be derived using the basic formula  $y = mx + b$ , where m equals the slope of the regression and b is set to 0:
  - Cumulative glucose consumption =  $m_1 \times \text{capacitance}$
  - Nuclei count =  $m_2 \times \text{cumulative glucose consumption}$
  - Nuclei count =  $m_3 \times \text{capacitance}$
- In the iCELLis Nano bioreactor, using equation 3 provides the most direct method of converting capacitance measurement to cell density
- By substituting equation 1 into equation 2, the following equation can be derived:
  - Nuclei count =  $m_2 \times m_1 \times \text{capacitance}$
- Further substitution of equation 3 into equation 4 yields
  - $m_3 = m_2 \times m_1$
- By substituting the regression slopes from Figure 2 into equation 5, we demonstrate:
  - $928.47 = 465201 \times 0.002$
  - $928.47 \approx 930.40$

### Translating iCELLis Nano bioreactor correlations to the iCELLis 500+ bioreactor

- Since nuclei counts are not possible in the iCELLis 500+ bioreactor, the relationship between capacitance and cell density can be triangulated using glucose measurements
- In the iCELLis 500+ bioreactor, we can create a new correlation between cumulative glucose consumption and capacitance to obtain the following:
  - Cumulative glucose<sub>500+</sub> consumption =  $m_{4_{500+}} \times \text{capacitance}_{500+}$
- The relationship between cell density and glucose consumption is expected and assumed to be the same in the iCELLis 500+ as in the iCELLis Nano bioreactors, therefore Equation 8 can be substituted into Equation 2 to obtain theoretical nuclei counts in the iCELLis 500+ bioreactor:
  - Nuclei count<sub>500+</sub> =  $m_2 \times m_{4_{500+}} \times \text{capacitance}_{500+}$

## CONCLUSION

- Aber's FUTURA biomass probe can be used to provide accurate, continuous readings of cell biomass while using the iCELLis bioreactor technology
- Cumulative glucose consumption, nuclei counts, and capacitance were successfully correlated in the iCELLis Nano bioreactor
- Once correlations are established in the iCELLis Nano bioreactor, it is possible to use iCELLis 500+ bioreactor capacitance measurements to calculate the cell density in the iCELLis 500+ bioreactor