Oncolytic Virus Production in iCELLis® Bioreactor

Amy Correia, Keen Chung, Deepika Vallabhaneni, Udara Dharmasiri, Amelia Baynham & Rachel Legmann • Pall Biotech, 20 Walkup Drive, Westborough, MA 01581, USA

INTRODUCTION

Immunotherapy clinical trials required high titer virus preparation to adequately deliver the therapeutic transgene. Viruses represent an attractive vehicle for cancer gene therapy due to their high efficiency of gene delivery. As demand for these new therapies increases, a robust and scalable manufacturing process is critical. The distribution of VERO cells and viral vector produced during growth and production phases across various scales of Pall's iCELLis fixed-bed bioreactor were studied. Comparison of homogeneity of various fixed bed heights confirm a robust and reproducible process across four different iCELLis Nano bioreactor scales, different fixed bed height and compaction. All four iCELLis Nano bioreactors were inoculated with VERO cells at a seeding density of 1.0 x 10^4 cells/cm^2. Similar doubling time was observed in all bioreactors: 30 – 33 hours. At the end of the growth phase, we have shown that the cells were equally distributed within all fixed-bed bioreactors. The solution to successful large-scale upstream envelope viral vector production for the immunotherapy market is dependent upon two crucial strategies: robust and reproducible cell growth and homogenous viral vector distribution across the fixed bed.

An oncolytic viral vector was used to investigate viral infection efficiency and the distribution of oncolytic virus in four different scales of iCELLis Nano bioreactors, low and high compaction.

Study results have clearly demonstrated uniform GFP (green fluorescent protein) labeled oncolytic virus distribution in all fixed-bed locations. An average of approximately 80% cell population on macro-carriers were positive for GFP, suggesting high infection efficiency. Further, viral titer evident by GFP intensity suggests an equal distribution of the produced viral vector among fixed-bed macro-carriers. This study indicates an even distribution of the cells and the viral vector in fixed-bed bioreactors. Present study results demonstrate the iCELLis Nano bioreactor can be used as a platform for scale-up envelope viral vector production in adherent cells.

OBJECTIVE

- Reproducible distribution of VERO cell growth and homogeneous oncolytic viral vector expression in the iCELLis Nano bioreactor

Growth of VERO Cells and Production of Oncolytic Virus Performed in Different Scales of iCELLis Nano Bioreactors

Table 1

Experimental study plan

	Case Study 1 Cell Distribution			Case Study 2 Viral Vector Distribution			
iCELLis Nano bioreactor run	1	2	3	1	2	3	4
Bioreactor scale (m^2)	0.53	1.07	2.65	0.53	0.80	2.65	4.00
Fixed bed height (cm)	2	4	10	2	2	10	10
Compaction level	Low	Low	Low	Low	High	Low	High
Seeding density (x10^4 cells/cm^2)	1.0	1.0	1.0	7.5	5.0	2.0	2.0
Growth phase period (day)	5	5	5	1	2	3	3
Production phase period (hours)	N/A	N/A	N/A	16	16	16	16
Fixed-bed sampling carrier location	3	3	3	5	5	5	5

MATERIALS & METHODS

Case Study 1: Cell Growth and Distribution

Materials & Equipment
- VERO cell line provided by case study 1 client
- DMEM + 10% FBS complete medium
- Microscope and hemocytometer
- NC-200♦ lysis buffer A (ChemoMetec, part number [P/N] P0820-5190)
- iCELLis Nano bioreactors: 0.53 m^2 (P/N 810039NS)
  1.07 m^2 (P/N 810061NS)
  2.65 m^2 (P/N 810206NS)

Methods
- Bioreactors seeded at 1.0 x 10^4 cells /cm^2
- Last day of growth 3 carriers were sampled for cell density from the top, middle, and bottom of the fixed bed

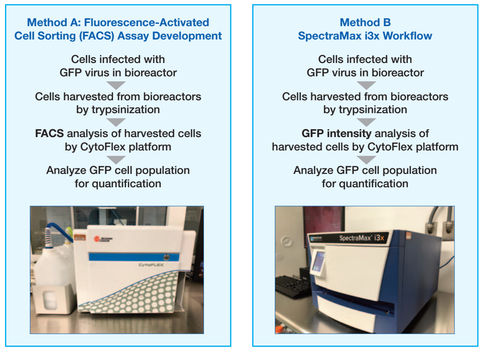
Case Study 2: Viral Vector Distribution

Materials & Equipment
- VERO cell line provided by case study 2 client
- DMEM + 10% FBS complete medium
- Microscope and hemocytometer
- NC-200 lysis buffer A (P/N P0820-5190)
- GFP-tagged virus
- iCELLis Nano bioreactors: 0.53 m^2 (P/N 810039NS)
  0.80 m^2 (P/N 810040NS)
  2.65 m^2 (P/N 810206NS)
  4.00 m^2 (P/N 810042NS)
- CytoFlex♦ flow cytometer platform (Beckman Coulter)
- SpectraMax♦ i3x platform (Molecular Devices)

Methods
- Cells infected with GFP-tagged virus at multiplicity of infection (MOI) 0.01
- Harvested at 16 hours. Post infection
- Carriers sampled at multiple locations for analysis of titer and vector distribution

Figure 1

Case study 2: Viral vector distribution methods

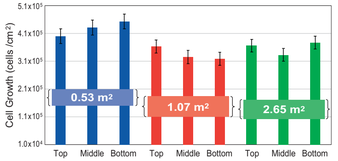


RESULTS

Case Study 1: Cell Growth and Distribution

Figure 2

VERO cell distribution during growth phase; N=3, three carriers were sampled from each location of all the iCELLis Nano bioreactors



Case Study 2: Viral Vector Distribution

Figure 3

Workflow for viral vector distribution in the fixed-bed iCELLis Nano bioreactor

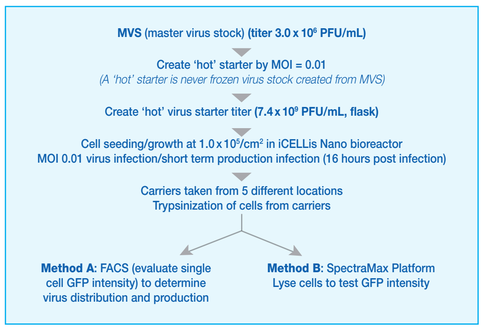
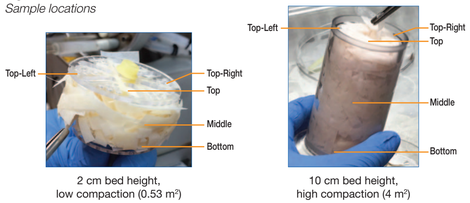


Figure 4

Sample locations

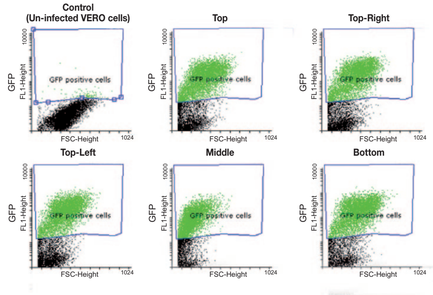


Case Study 2: Viral Vector Distribution *(Continued)*

Homogeneous Distribution of Envelope Viral Vector in a Highest Scale iCELLis Nano Bioreactor - Method A Results

Figure 5

Representative virus distribution evident by GFP-labelled cells in 4 m^2. Similar distribution was observed in 0.53 m^2, 0.80 m^2 and 2.65 m^2 respectively.



Viral Vector Distribution in iCELLis Nano Bioreactor - Results

Figure 6 - Method A

Quantification of virus distribution evident by GFP-labelled cells

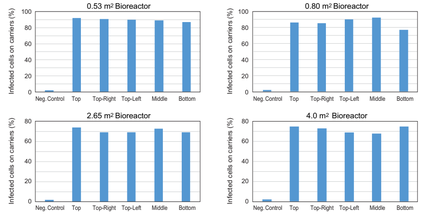
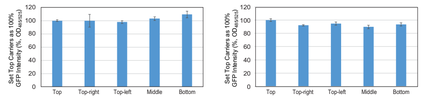


Figure 7 - Method B

Quantification of virus production evident by GFP intensity (virus positive)



CONCLUSION

Case Study 1: Cell Growth and Distribution

- Comparison of homogeneity of various fixed bed heights confirm a robust and reproducible process across three bioreactor scales and three fixed bed heights
- Figure 2 prominently demonstrates robust and reproducible cell growth in the iCELLis Nano bioreactor
- All three iCELLis Nano bioreactors grew equivalently with a doubling time in the iCELLis Nano bioreactor between 30.9 to 33 hours respectively. There was also very little difference across all three size iCELLis Nano bioreactors and the carrier level was less than 1.0 standard deviation with regards to nuclei count

Case Study 2: Viral Vector Distribution

- Pall's Accelerator℠ process development services (PDS) team, successfully developed analytical methods to test viral vector expression distribution in iCELLis Nano fixed-bed bioreactor
  - FACS results indicates homogenous virus distribution 16 hours post infection. There is negligible difference across all four size iCELLis Nano bioreactors, suggesting robust virus distribution and production
  - Quantification of virus distribution results (Figure 6) marked by GFP cells (virus positive): Overall, 70% to 90% cells were GFP positive 16 hours post infection, suggesting robust virus infection and production. Negligible difference of GFP distribution across carriers in bioreactors.
  - Quantification of virus production (Figure 7) marked by GFP intensity (virus positive): Negligible difference of GFP intensity across carriers within bioreactors suggesting robust viral production.
  - Robust and homogenous virus distribution in both high and low compaction iCELLis Nano Bioreactors tested
- iCELLis Nano bioreactors provide uniform viral infection and production both in low and high fixed-bed compaction
- Present study results demonstrate the iCELLis Nano bioreactor can be used as a platform for scale-up envelope viral vector production in adherent cells

Contact: +1 800 717 7255 (USA) • +41 (0)26 350 53 00 (Europe) • +65 6389 6500 (Asia/Pacific) • Email us at: www.pall.com/contact • Web: www.pall.com/biotech