Chemically Defined Upstream and Downstream Lentivirus Production for High T Cell Transduction

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Background and Introduction

Clinical success of viral vectors such as lentivirus for CAR-T lymphocyte cancer therapy demonstrate not only the efficiency of this promising therapeutic, but also highlight the need to manufacture increasing amounts of lentivirus proficiently and with reduced cost. Manufacturing of lentivirus for gene and cell therapies typically includes animal components such as fetal bovine serum (FBS) for cell growth and viral vector production. However, animal component inclusion represents a regulatory hurdle that governing agencies are demanding to be removed. In addition, high amounts of FBS can be expensive at scale and can negatively impact production times, viral titers, and downstream purification. Therefore, to overcome these constraints, we have optimized lentivirus production in the iCELLis® Nano Bioreactor in completely animal-component free conditions to yield high titer lentivirus for use in CAR-T manufacturing.

InVitria's OptiPEAK HEK293t®

- Animal-component free media formulated with recombinant proteins for adherent HEK cells.
- Demonstrates faster doubling times and eliminates variability associated with the use of serum.
- Produces higher lentivirus titer in the iCELLis Nano Bioreactor when compared to serum-containing medium.[1]

Pall's iCELLis Nano Bioreactor

- Provides clinical and commercial manufacturing with adherent cells.
- Controls pH and dissolved oxygen (DO) through a falling film that provides high gas transfer without sparging and no cell shear due to bubble burst.
- Exhibits bench and commercial size scalability by maintaining fixed-bed height and carrier compaction.

InVitria's Optiferrin®

- Optiferrin is a recombinant human transferrin from a non-mammalian expression system.
- Increases transfection efficiency and viral titer in transfection complexes in both flatware and the iCELLis Nano bioreactors.[2]

Methods

iCELLis Nano Bioreactor Conditions for Lentivirus Production

- 0.53 m^2 iCELLis Nano bioreactors were batched with OptiPEAK HEK293t media.
- iCELLis Nano bioreactors were inoculated with HEK293t that were expanded in OptiPEAK HEK293t for three passages out of cryopreservation. Bioreactors were seeded at a density of 10,000 cells per cm^2.
- iCELLis Nano bioreactors were fitted with an Aber biomass Probe for online monitoring of cell growth and density. Online measurements for pH, dissolved oxygen (DO) and temperature were recorded with BioXpert software. Media samples from the bioreactors were pulled daily for offline measurement of pH and glucose.

iCELLis Nano Bioreactor Transfections

- 48 hours post inoculation, bioreactors were transfected with second generation lentivirus plasmids for production of VSV-G pseudotyped lentivirus carrying a bicistronic CD19-CAR with CD3ζ stimulatory domain and P2A-GFP to rapidly test for functional titer (A kind gift from Scott McComb, Addgene Plasmid #135992).[3]
- Transfection complexes were formed using OptiPEAK HEK293t basal medium with or without 1 mg/mL Optiferrin in a volume of 10% of the working volume of the vessel. PEIpro® or FectoVIR®-AAV (Polyplus-Transfection) were added at a ratio of 1 µg DNA to 1.5 µL reagent. The total amount of plasmid DNA was calculated as 0.24 µg DNA per cm^2. Transfection complexes were added to the bioreactors without a media change, and 3 hours later a complete media change was performed to the production media. Virus production media was basal OptiPEAK T-lymphocyte XPR® media supplemented with 1x ITS-E® (InVitria) and 250mg/L Cellastim S® (InVitria).

Table 1

iCELLis Nano Bioreactor Set Points Pre- and Post- Transfection

iCELLis Nano Bioreactor Set Points	Pre-transfection	Post-transfection
Linear speed	2	2
DO	95%	55%
pH	7.25	6.8

Lentivirus Concentration and Functional Titering

- 48 or 72 hours post transfection lentivirus was harvested from the iCELLis Nano bioreactors. Functional titer was determined with an HT-1080 transduction assay and transduced cells were analyzed with flow cytometry.
- Lentivirus concentration was performed with tangential flow filtration (TFF) using Vivaflow® 50 filtration cassettes with 100k MWCO (Sartorius Corporation). Cassettes were washed and equilibrated with 10mM Tris-HCl buffer, pH 7.8 with or without Cellastim S. Diafiltration was performed with Tris-HCl buffer plus 200 mM NaCl without Cellastim S.
- T cells were transduced at an MOI of 10 in combination with LentiBOOST® (Sirion Biotech). Mock and transduced T cells were expanded in OptiPEAK T lymphocyte XPR.

Results

Optiferrin Increases Titer in the iCELLis Nano Bioreactor for CD19 CAR Lentivirus Production

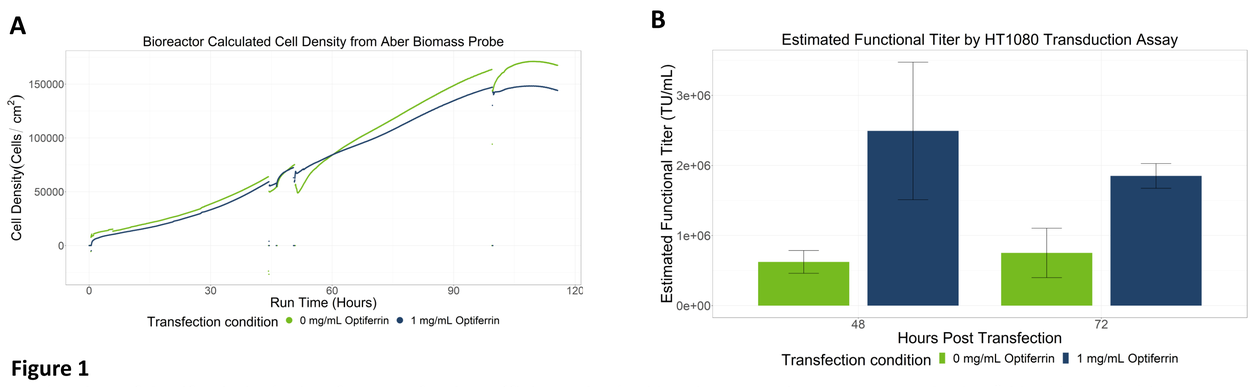


Figure 1

Evaluation of Optiferrin inclusion for transfection efficiency in the iCELLis Nano Bioreactor. Two 0.53 m^2 iCELLis Nano bioreactors were transfected with PEIpro either with 1 mg/mL Optiferrin in the complexing medium, or 0 mg/mL Optiferrin in the complexing medium. ***(A)*** *Growth curves from cell density captured online with the Aber biomass probe. Cell density was calculated from the capacitance measurements. Bioreactors were transfected 48 hours post inoculation.* ***(B)*** *Functional titer from lentivirus produced in the iCELLis Nano bioreactor with transfection conditions that either included 1 mg/mL Optiferrin or 0 mg/mL Optiferrin in the complexing medium. Virus supernatant was harvested from the iCELLis bioreactors at 48 hours and 72 hours post transfection. Functional titer was determined by HT1080 transduction assay. Error bars represent standard error of technical replicates.*

Optiferrin and High-Performance Transfection Reagents Increase Titer in the iCELLis Nano Bioreactor

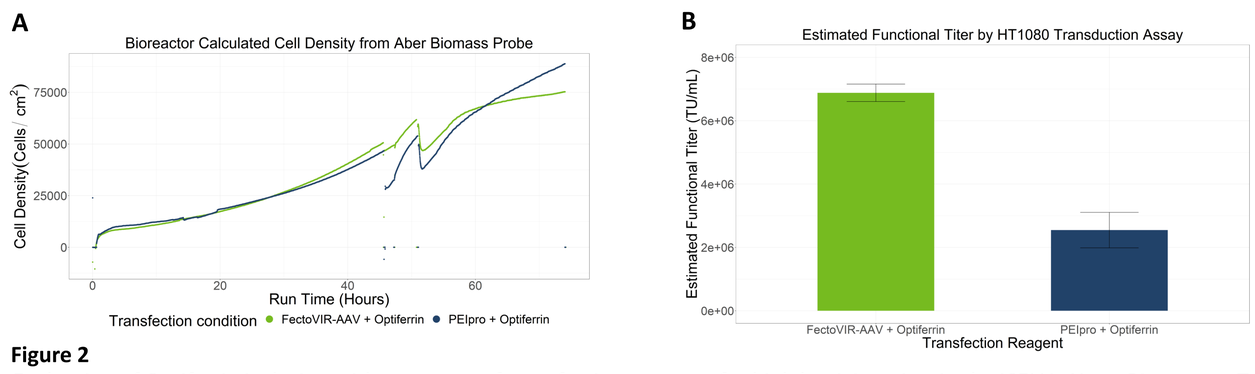


Figure 2

Evaluation of Optiferrin inclusion with two types of transfection reagents for high lentivirus titer in the iCELLis Nano Bioreactor. Two 0.53 iCELLis Nano bioreactors were transfected with either PEIpro or FectoVIR-AAV in combination with 1mg/mL Optiferrin in the complexing medium. ***(A)*** *Growth curves from cell density captured online with the Aber biomass probe. Cell density was calculated from the capacitance measurements. Bioreactors were transfected 48 hours post inoculation.* ***(B)*** *Functional titer from lentivirus produced in the iCELLis Nano bioreactor with transfection conditions that either included 1 mg/mL Optiferrin with PEIpro or 1 mg/mL Optiferrin with FectoVIR-AAV in the complexing medium. Virus supernatant was harvested from the iCELLis Nano bioreactors at 48 hours post transfection. Functional titer was determined by HT1080 transduction assay. Error bars represent standard error of technical replicates.*

Inclusion of Cellastim S During Downstream Processing Increases Recovery for CAR-T Production

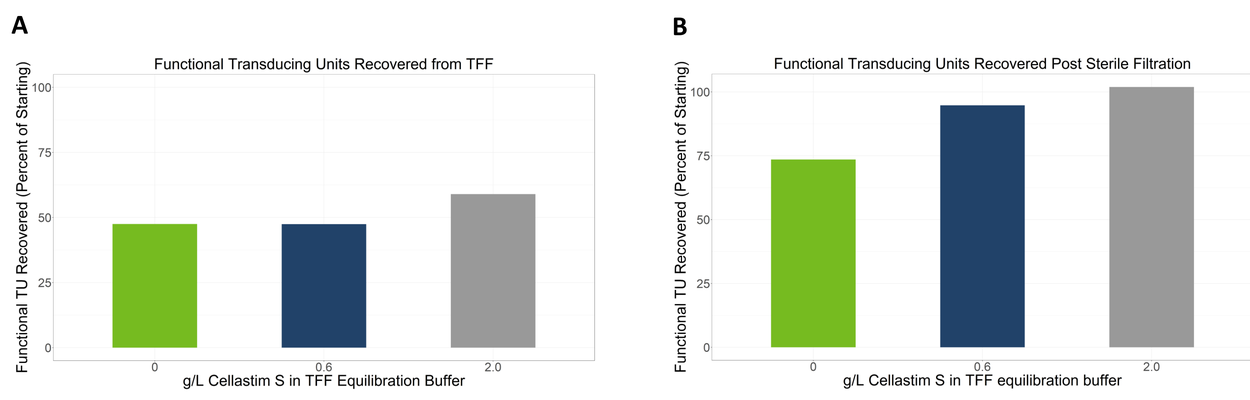
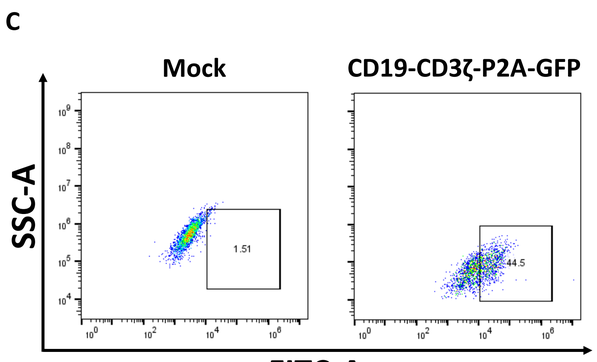


Figure 3

Downstream concentration with Cellastim S and analysis of T cell transduction. Virus supernatant from one bioreactor run produced with PEIpro and Optiferrin was concentrated with TFF. ***(A)*** *Percentage of functional transducing units (TU) recovered post TFF. r-Albumin (Cellastim S) was added to the equilibration buffer at 0, 0.6, or 2 g/L. Recovery was calculated as total TU over starting total TU.* ***(B)*** *Recovery of functional TU post sterile filtration of the TFF concentrate. X-axis represents g/L of Cellastim S added in the equilibration buffer at the start of TFF concentration. Percent recovery was calculated as total TU post sterile filtration over total TU post TFF recovery.* ***(C)*** *Evaluation of transduction efficiency with concentrated lentivirus. PBMC was isolated from one healthy donor and activated T cells were transduced 24 hours post activation.*



Conclusions

- **The inclusion of Optiferrin produces the most significant increase in viral titer, with a nearly 6-fold increase in viral titer with FectoVIR-AAV compared to transfection complexes with PEI-pro that did not contain Optiferrin. Including Optiferrin with PEIpro increased viral titer almost 3-fold compared to PEIpro without Optiferrin. Final optimization of the transient transfection conditions resulted in lentivirus titers of approximately 7e+06 TU/mL pre-concentration with a viral vector for CAR-T production.**
- **We have demonstrated a manufacturing process that is animal component free, with high titer production and downstream recovery for CAR-T manufacturing for more efficient vector manufacture.**
- **Inclusion of recombinant albumin in the equilibration buffer added to a tangential flow cassette further increased downstream recovery of the lentivirus, increasing recovery approximately 15% compared to no albumin, and further recovering 100% of the lentivirus functional vectors during sterile filtration. The concentrated lentivirus produced in animal component free conditions is highly functional, and capable of high efficiency transduction of primary T lymphocytes, with transducing over 40% of activated T cells.**

References
1. Pezoa, SA, Alfano, R, Pennybaker, A, Hazy, N. (2021) Efficient lentiviral vector production in a chemically defined, blood-free and serum-free medium, scalable to the iCELLis Bioreactor Technology, [Poster, *ASGCT]. www.invitria.com/recources/lentiviral-vector-production-icellis-bioreactor/*
2. Pezoa, SA, Alfano, R, Hazi, N. (2022) Evaluation of transfection reagents for high titer viral vector production in the iCELLis Nano bioreactor, Poster, *ISCT.*
3. Bloemberg D, et al. (2020) A High-Throughput Method for Characterizing Novel Chimeric Antigen Receptors in Jurkat Cells. *Mol Ther Methods,* 16:238-254.

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