Evaluation of Transfection Reagents for High Titer Viral Vector Production in the iCELLis® Nano Bioreactor

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BACKGROUND AND INTRODUCTION

The Pall iCELLis Nano bioreactor is a scalable fixed bed bioreactor technology commonly used for the production of viral vectors and vaccines. We have demonstrated that OptiPEAK® HEK293t, a serum-free and animal component-free media developed for HEK cells, improves cell doubling time as well as lentivirus titer over traditional serum-based media in the iCELLis Nano bioreactor[1]. However, per cm^2, productivity was still lower when compared to flatware.

Prior to this work, different types of transfection reagents had not been evaluated for gene expression comparison in the iCELLis bioreactor. We hypothesized that traditional reagents like polyethyleneimine (PEI) are less efficient in the iCELLis Nano bioreactor, and that reagents optimized for suspension would improve productivity.

InVitria's OptiPEAK HEK293t

- A chemically defined media for adherent HEK, and optimized for viral vector production in the iCELLis bioreactor
- Formulated with recombinant proteins from a non-mammalian expression platform
- Eliminates variability and safety concerns surrounding the use of serum
- Produces higher titer lentivirus in the iCELLis Nano bioreactor when compared to serum-containing media

InVitria's Optiferrin®

- Optiferrin is a recombinant human transferrin from a non-mammalian expression system
- The biological activity of Optiferrin is equal to human serum transferrin, and is endocytosed and recycled at equivalent rates

Pall's iCELLis Nano Bioreactor

- Provides clinical and commercial manufacturing of adherent cells products by utilizing:
  - Cell substrate composed of medical grade, uncoated, uncharged polyethylene terephthalate (PET) carriers
  - A closed system with reduced footprint and minimal aseptic handling
- Controls pH and dissolved oxygen 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

METHODS

Flatware Cultures and Transfections

- Cells were seeded at 50,000 cells per cm^2 24 hours before transfection
- GFP reporter plasmid DNA was delivered at 0.2 µg plasmid per cm^2 and complexed with 10% of the vessel working volume. Cells were incubated for 48 hours and then harvested and analyzed by flow cytometry

iCELLis Nano Bioreactor Cultures and Transfections

- 0.53 m^2 iCELLis Nano bioreactors were inoculated with HEK293T cells at a density of 10,000 /cm^2. The bioreactors were batched with OptiPEAK HEK293t medium
- All bioreactors were transfected on day 2 post-inoculation. A cell density of 60,000 cells per cm^2 was targeted for transfection based on previous results[2] using Aber's biomass probe to track cell density
- The transfection mixture complexation volume was 10% of the total bioreactor working volume
- A single GFP plasmid was used to determine fluorescence intensity. Bioreactors were transfected with 0.2 µg plasmid per cm^2
- Optiferrin was included at 1 mg/mL in the complexation with PEIpro♦ and FectoVIR♦-AAV reagents[3] but not the liposome reagent due to uncertainty about performance in the bioreactor
- Two days post-transfection, iCELLis bioreactor carrier strips from the top, middle, and bottom of the fixed bed were removed from the bioreactors and lysed to measure fluorescence and total protein. Total fluorescence intensity was measured with a BioTek fluorescence plate reader, and protein content was measured by Bradford assay. As a measure of transfection efficiency, GFP fluorescence intensity was normalized to total protein for each sample

Table 1

iCELLis Nano bioreactor set points pre- and post-transfection

iCELLis Nano Bioreactor Set Points	Pre-Transfection	Post-Transfection
Dissolved oxygen	95%	55%
pH	7.25	7.00
Media linear speed	2 cm/s	2 cm/s
Temperature	37 °C	37 °C

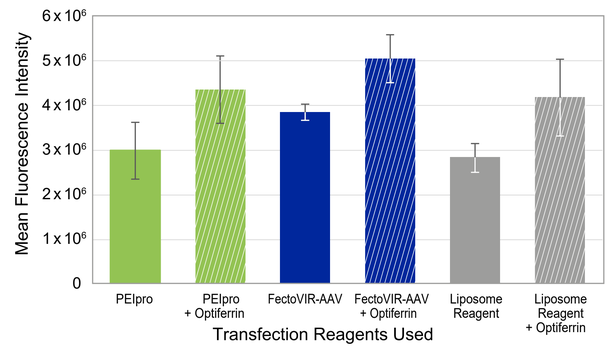
Pall's iCELLis Nano Bioreactor

RESULTS

Inclusion of Optiferrin Increases GFP Fluorescence Intensity in Flatware

Figure 1

GFP fluorescence intensity in flatware. HEK293T were transfected with three different transfection reagents in flatware. Mean fluorescence intensity is measured by flow cytometry. Error bars represent standard deviation of two technical replicates for one biological replicate. Inclusion of Optiferrin increased GFP fluorescence intensity when combined with the three reagents tested.

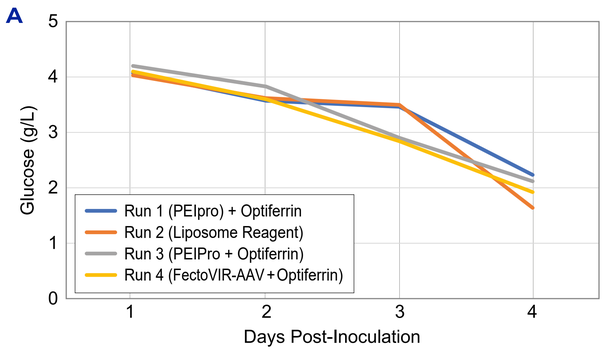


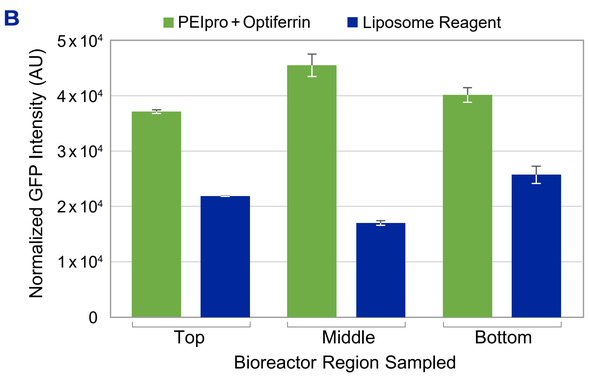
Transfection Reagents Produce Different GFP Expression in iCELLis Nano Bioreactors

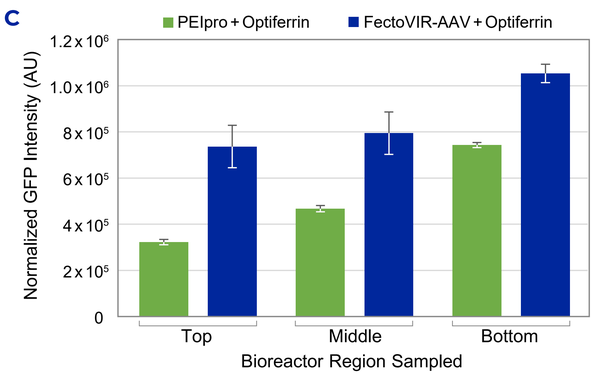
Three different transfection reagents were tested in two sets of two iCELLis Nano bioreactor runs (PEIpro vs. liposome reagent, PEIpro vs. FectoVIR-AAV). Growth trends and glucose consumption between the four bioreactor runs were similar. FectoVIR-AAV displayed the overall highest normalized GFP fluorescence intensity and least amount of heterogeneity between the three regions of the fixed bed. The liposome-based reagent produced the lowest normalized GFP fluorescence intensity.

Figure 2

Evaluation of transfection reagents in the iCELLis Nano bioreactor. ***(A)*** *Glucose consumption of the four iCELLis Nano bioreactor runs in OptiPEAK HEK293t.* ***(B)*** *Normalized GFP fluorescence intensity from lysed iCELLis bioreactor carrier strips transfected with PEIpro or the liposome reagent* ***(C)*** *Normalized GFP fluorescence intensity from lysed iCELLis carrier strips transfected with PEIpro or FectoVIR-AAV. In runs 3 and 4 (A and C), media was not changed prior to transfection. In both (B) and (C), total GFP fluorescence was measured and normalized to total protein. Y-axis shows fluorescence in arbitrary units (AU). Error bars represent standard deviation of 2 technical replicates.*







CONCLUSIONS

- **Specific types of transfection reagents perform differently in the iCELLis Nano bioreactor. FectoVIR-AAV, developed for suspension cultures, showed a 2-fold increase in fluorescence intensity over PEIpro in the iCELLis Nano bioreactor, suggesting this type of reagent could be used for higher viral titer in the iCELLis bioreactor**
- **Transfection reagents that are based on liposomes may be more susceptible to degradation in the dynamic environment of the iCELLis bioreactor, or perhaps the addition of Optiferrin may have helped**
- **The inclusion of Optiferrin increased gene expression for all transfection reagents in flatware**
- **Transfection efficiency in OptiPEAK HEK293t serum-free, animal component free media may be higher without a medium exchange immediately before transfection**

References

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