Simpler, Faster, Cheaper Advanced Therapy Manufacturing With Intensified Lentiviral Vector Production Using TFDF®-Based Perfusion Cell Culture

Application Note

Summary

The development of new chimeric antigen T cells (CAR-T) and other cell therapies where lentiviral (LV) transduction is a key component increases the demand for these viral vectors. Improving the efficiency of current low yield lentivirus manufacturing is imperative to meet this demand.

In this study, a >10-fold increase in production of active LV from stable producer cells was achieved from cell culture intensification using the TFDF® perfusion technology compared to a standard batch process at 2 L bioreactor scale. The intensified process enabled a high cell specific virus productivity, and the virus produced was able to be continuously harvested to cold storage to maintain high functionality.

Taking a CAR-T blood cancer therapy as a representative example, a TFDF-intensified process at 10 L bioreactor scale would be enough to quickly produce the required amount of active LV particles for T-cell transduction before expansion for typical Phase II or III clinical trials with one hundred patients, instead of having to scale up to a 200 L bioreactor for a typical batch process. Process intensification results in significant cost saving by producing 10-fold more doses per batch with a smaller bioreactor footprint and reduced reagent usage. These benefits will greatly facilitate and accelerate the commercialization of CAR-T and other advanced therapies.

Introduction

Chimeric antigen receptor T cells (CAR-T) therapies offer substantial potential for treating conditions like hemoglobinopathies, immunodeficiency, and various forms of cancer. In these therapies, viral vectors are a crucial component in the creation of the final CAR-T product. Viral vectors facilitate the delivery of the gene of interest, which helps T cells recognize cancer cells. Lentiviruses are often chosen as the viral vector due to its effectiveness in transducing dividing and non-dividing cells and its established safety profile (Sinn et al., 2005). With over 200 ongoing clinical trials using LV for either ex vivo cell modification or in vivo therapy, and recent FDA approval of several ex vivo LV therapies, a major surge for viral vector demand is happening (clinicaltrials.gov). Low lentivirus titer production achieved from current cell cultures require the use of large production volumes to meet the demand, implying large-scale media preparation and product storage, as well as long and complex bioreactor seed trains and virus manufacturing processes. There is also the challenge posed by the labile nature of LV with the particles having a half-life of 8 – 40 hours at room temperature and 8 – 12 hours at 37°C, which can further reduce effective titer (Dautzenberg et al., 2021, Labisch et al., 2021, and Higashikawa and Chang 2001). One way to resolve these large-scale manufacturing drawbacks is to intensify the LV production process.

Recent publications have shown that process intensification, using the Tangential Flow Depth Filtration (TFDF) perfusion technology for LV manufacturing increases process productivity and lowers costs (Tran et al., 2022; Tona et al., 2023). Moreover, TFDF perfusion enables continuous harvest of the LV directly to a storage vessel at 4°C over a period of 72 hours to maintain LV functionality titer (Labisch et al., 2021). This continuous gentle harvest feature of TFDF remedies the LV inactivation issue, which negatively impacts LV production at batch mode. TFDF technology offers a 2 – 5 µm pore size depth filter, operated in tangential flow mode, to retain cells and cell debris in the bioreactor, while enabling waste metabolites and secreted vector to be continuously harvested.

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In this application note, LV vectors were produced from a LV stable producer cell line at 2 L bioreactor scale using a batch culture as a control in comparison to a TFDF-based perfusion cell culture strategy. An over 10-fold higher production of active virus was achieved with the TFDF-intensified process. The result can be applied as an example of clinical manufacturing sufficient to supply the material needed for a Phase II or III CAR-T trial. For such a clinical trial, 100 patients would be recruited, and a total amount of 5×10^{11} transduction units (TU) of LV vector would be required for T-cell transduction. A 10 L TFDF-intensified cell culture process was calculated to be sufficient to produce enough LV vector for such a CAR-T clinical trial example. The execution of a 10 L TFDF-intensified cell culture process scale-up was comparable to the performance seen at the 2 L scale. The LV production from the 10 L TFDF-based process is equivalent to a 172 L batch process needed to meet the demand for this clinical trial scenario. Here, TFDF-based intensified LV production provides solutions to large-scale pain points, including the cost of major cell culture consumables (media, endonuclease), the estimated manufacturing footprint, and process preparation (seed train).

Materials and Methods

Cell Cultures

A vial containing HEK 293SF-LVP clone #3E9 cells with GFP as the transgene, courtesy of the National Research Council Canada (NRC-CNRC) (Broussau et al, 2023), was thawed and subcultured until the viability (≥95%) and doubling time (≤30h) were stable. The cell culture incubator conditions were set at 37°C, 8% CO_2, 125 ± 5 rpm (with 19 mm shaking diameter). The seeding density target was 0.3 – 0.8×10^6 viable cells/mL for inoculum preparation. HyCell TransFx-HTM (Cytiva, cat# SH30939.02) or BalanCD HEK293 media (Fujifilm Irvine Scientific, cat# 94137-10L) was used for cell passaging, seed train, and virus production. Viable cell density (VCD), cell viability, and nutrient metabolites analysis were performed using a BioProfile® Flex2 Analyzer (Nova® Biomedical).

Bioreactor Intensification

BioBLU® 3c Single-Use Vessels with macrosparger and two pitched-blade impellers (Eppendorf®, cat# 1386121000) and BioBLU 10c Single-Use Vessels with macrosparger and one pithed-blade impeller (Eppendorf, cat#1386140000) were used with BioFlo® 320 Controllers (Eppendorf) for cell expansion and virus production. To maintain equivalent power input, agitation was set at 200 rpm for 2 L and 175 rpm for 10 L working volume. Temperature was set at 37°C, pH was set at 7.0 ± 0.2 controlled with CO_2, and dissolved oxygen (DO) was set at 50% controlled with air and O_2. To maintain equivalent gas transfer rate, the total constant gas flow rate was set at 0.1 – 0.3 L/min for 2 L and 0.6 –1.0 L/min for 10 L bioreactors. Gases were supplied via the macrosparger using the 3-gas auto mixture of air, O_2, and CO_2. Each perfusion and control batch bioreactor ran in triplicate for reproducibility.

The seeding density for batch processes targeted ~0.4×10^6 viable cells/mL for inoculation. The seeding densities for the TFDF bioreactors varied between 0.4×10^6 and 1.2×10^6 cells/mL due to timing alignment between batch control bioreactor and perfusion production bioreactor. Both processes used HyCell TransFx-H media. The 2 L TFDF-based perfusion bioreactors were achieved using the KrosFlo® TFDF Lab System (Repligen, cat# TFDFLP2S2F1T0NCFRS) equipped with a TFDF-30 ProConnex® TFDF Flow Path (Repligen, cat# STFDFCL15546S). The 10 L TFDF-based perfusion bioreactor was achieved using the KrosFlo TFDF Lab System equipped with a TFDF-150 ProConnex TFDF Flow Path (Repligen, cat# STFDFCL15112S) to maintain the same perfusion filter capacity of 66 mL culture per 1 cm^2 TFDF surface area during scalability when used in a perfusion mode and continuous clarification during production phase. For the execution of the TFDF-intensified process scale up, 2 L and 10 L perfusion bioreactors, using BalanCD media, were inoculated in parallel at 0.6×10^6 cells/mL with >95% viability. In a separate study, the use of BalanCD media resulted in comparable titers to that of HyCell TransFx-H and was shown to be more economical for the TFDF process (data not shown). The perfusion started at an initial flow rate of 1 vessel volume per day (VVD) when the VCD reached ~2×10^6 cells/mL and increased to 2 VVD when the VCD reached ~10×10^6 cells/mL. The scale up intensified runs (2 L vs. 10 L) were the exception, where the perfusion rate remained at 1 VVD. These perfusion strategies remained in place post-induction during the viral vector production process, along with daily sampling to monitor VCD, viability, metabolites, and post-induction LV harvesting.

Induction for LV Production

HEK 293SF-LVP clone #3E9 cells were induced with 10 nM coumermycin (Promega, cat# C9451) and 80 µg/mL cumate (Sigma-Aldrich, cat# 268402) for lentivirus production. At the 2 L scale, cells were induced when the VCD reached ~3 x10^6 cells/mL for batch and ~10 – 13 x 10^6 cells/mL for TFDF-intensified bioreactors. For the scale up process, both the 2 L and 10 L cultures were induced at ~9 x 10^6 cells/mL. To maintain induction in perfusion mode, perfusion media was spiked with inducers to a concentration of 10 nM coumermycin and 80 µg/mL cumate. At 16 – 22 hours post induction, the bioreactor and perfusion media received a bolus of sodium butyrate to a concentration of 7 mM (Sigma, cat# B5887) to enhance LV production. The inducers and sodium butyrate concentrations were maintained until the end of the production process.

Monitoring of LV Production

The production of LV was monitored daily for 5 – 7 days post-induction by taking samples from the bioreactor, TFDF permeate line, and harvest bottle. Samples were centrifuged for 10 min at 500 g, and only supernatant was kept for analytical assay. Transducing unit (TU) titration for LV was performed via a cell-based potency assay. HEK 293A cells were seeded into a 24-well plate at 1 x 10^5 cells/mL and 1 mL of viral dilutions were added to the plates. A spin inoculation was performed via centrifugation at 2500 rpm for 90 min. The cells were then incubated for 48 h post transduction and harvested for Fluorescence-Activated Cell Sorting (FACS) cytometry using Beckman CytoFLEX Flow Cytometer. Using Beckman CyExpert™ software, the cell populations were gated for GFP positive cells, and the percentage of GFP positive cells was calculated. This percentage was then used to back-calculate titer (TU/mL) using the equation below.

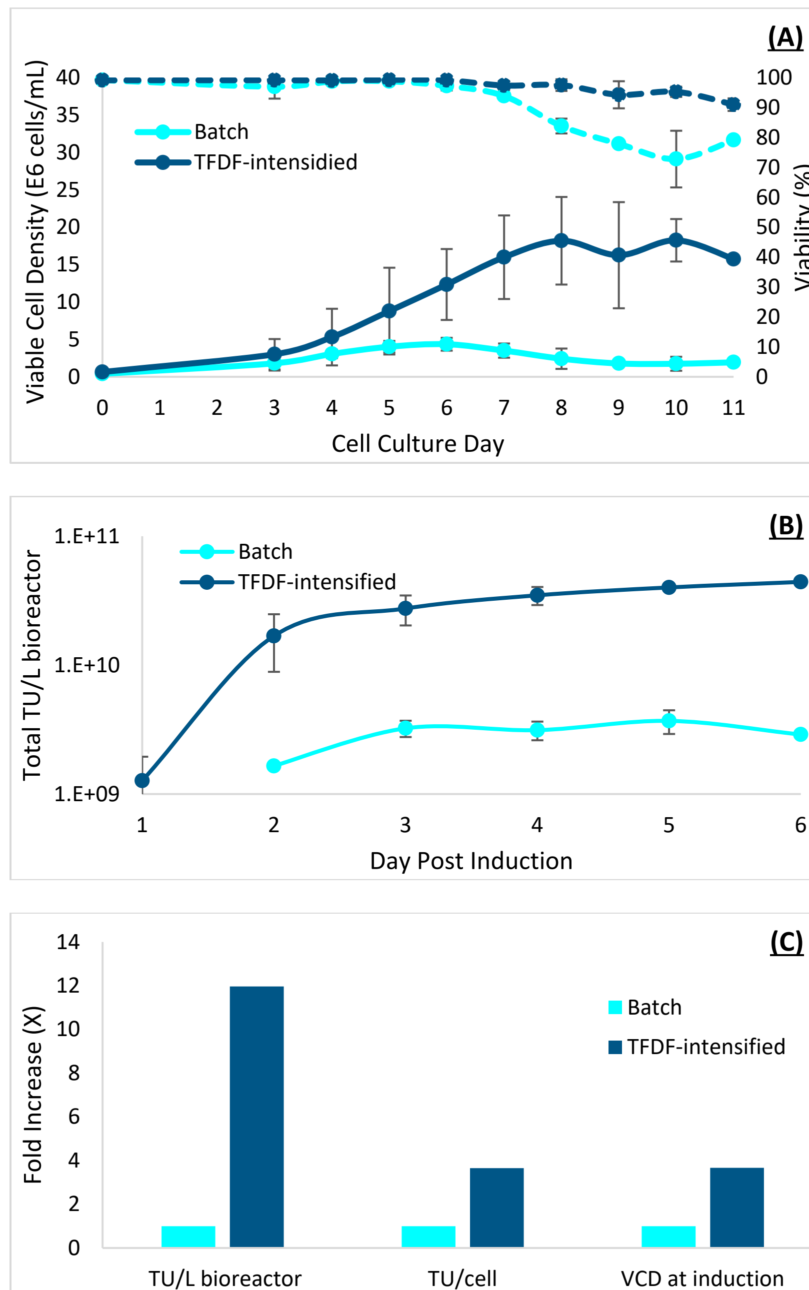
$$Titer\left(\frac{TU}{mL}\right) = \frac{\%\ of\ GFP\ positive\ cells\ \div 100}{viral\ volume} \times dilution\ factor\ \times cell\ number\ at\ time\ of\ transduction$$

Results and Discussion

Intensification of LV Production with TFDF Perfusion Technology

Cell culture intensification has been proven to significantly increase LV production compared to traditional batch processes (Tran et al., 2022; Tona et al., 2023). We used TFDF-based perfusion cell cultures to intensify LV production compared to batch cell culture processes at 2 L bioreactor volume scale. Figure 1A shows the average cell growth and viability data for the triplicate batch and TFDF-intensified bioreactor cell culture runs. In batch mode, the VCD reached ~3 x 10^6 cells/mL on day 4. In TFDF-based perfusion mode, VCD was enabled to reach ~9 x 10^6 cells/mL compared to batch bioreactors (~4 x 10^6 cells/mL) on cell culture day 5. The peak VCD are ~18 x 10^6 and ~4 x 10^6 cells/mL, respectively. After induction, the VCD in TFDF-based cell cultures continued to increase and remained elevated throughout the production process. Meanwhile, the VCD in batch mode maintained ~4 x 10^6 cells/mL for several days, then rapidly decreased to ~2 x 10^6 cells/mL. Higher viability was also observed throughout the production phase for TFDF-intensified bioreactors compared to batch bioreactors. At the end of the production, viability was 91% and 79%, respectively. The total TU/L of bioreactor in batch and TFDF-intensified bioreactor were 2.9 x 10^9 and 4.4 x 10^{10}, respectively (Figure 1B). The TFDF-intensified bioreactor runs enabled an increase of ~4-fold VCD at the time of induction, >10-fold in total TU/L of bioreactor for production, and ~4-fold TU/cell for productivity compared to batch process (Figure 1C).

Figure 1. Enhanced Production of LV From TFDF-Intensified Perfusion Cell Culture Compared to Batch Cell Culture



Data averaged from three batch vs three TFDF-intensified bioreactor cell culture runs (error bars: +/- standard deviation). (A) Cell growth and viability. (B) Total lentivirus TU production. (C) Fold increase of LV titer TU/L bioreactor, specific productivity TU/cell, and VCD at induction.

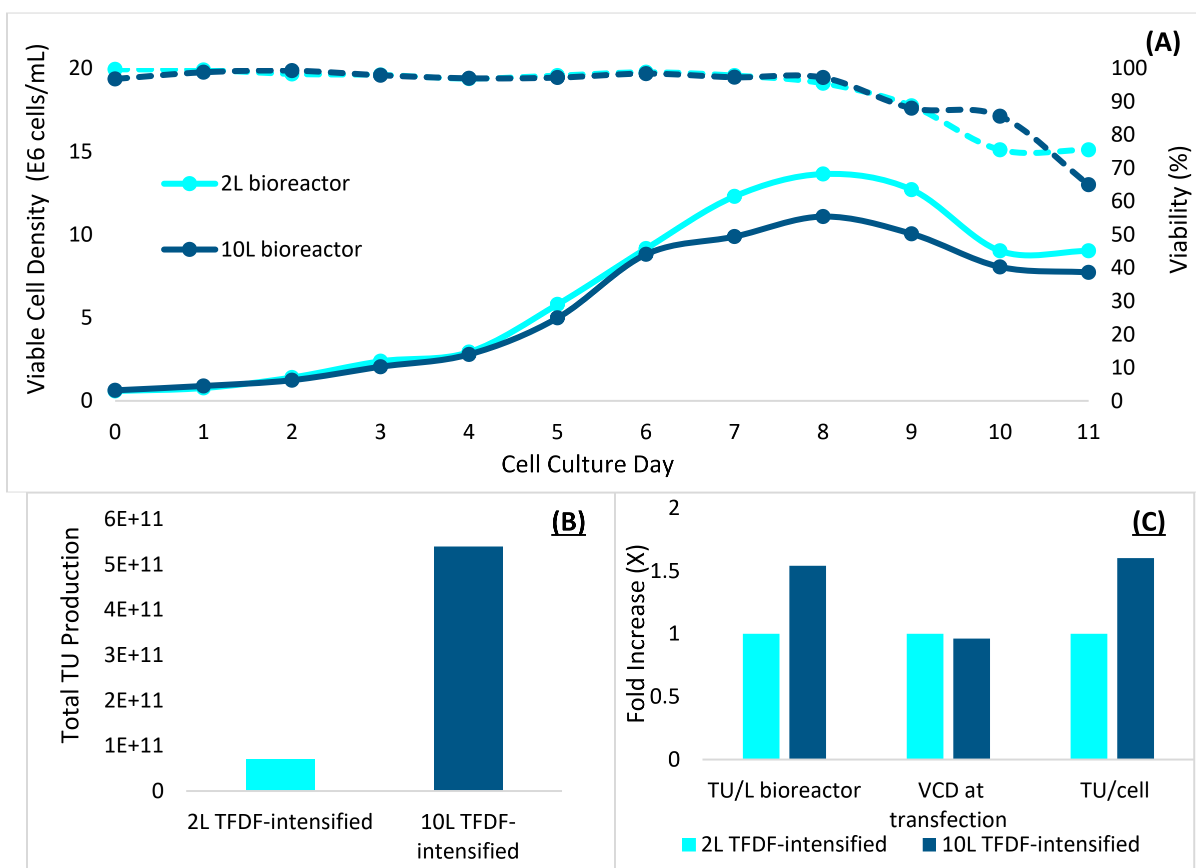
Scale Up LV Production for CAR-T Clinical Trial Example Scenario

Considering a Phase II/III clinical trial manufacturing scenario for a CAR-T therapy as an example, a minimum of 5×10^{11} total TU is typically needed to dose 100 patients (assuming a 20% recovery yield from bioreactor production to formulated LV and transduction with 1×10^{9} TU per CAR-T dose). Taking the performance of the batch process for LV production at 2 L scale, 172 L of batch cell culture would be required to meet the production demand. Given the same scenario and leveraging the ability to intensify

production by >10X with TFDF technology, a 10 L bioreactor run would be sufficient. The scale-up of the TFDF-intensified process from 2 L to 10 L was, therefore, executed to demonstrate that feasibility.

For the scale up of LV production, 2 L and 10 L TFDF-based perfusion bioreactor cell cultures were conducted in parallel and cell growth and viability data are presented in Figure 2A. At the time of induction, TFDF-based perfusion enabled a VCD of ~9 x 10^6 cells/mL with >95% viability on day 6 of cell culture. After induction, cell culture VCD of both bioreactors sustained ≥8 x 10^6 cells/mL with high viability, >75% and >65%, respectively for the 2 L and 10 L runs, until the end of the production process (5 days post induction). The total LV production at the end of the production process from the 2 L and 10 L bioreactor runs were 7.0 x 10^{10} and 5.4 x 10^{11} TU, respectively (Figure 2B). The 5-fold scale-up from 2 to 10 L resulted in a >7-fold increase in lentivirus production and ~1.5-fold increase in cell specific productivity (Figure 2C). The LV production performance achieved from the 10 L TFDF-intensified process confirmed that enough LV active particles would be produced to meet the demand for the CAR-T clinical trial example considered above.

Figure 2. LV Production Scale-up From 2 L to 10 L With TFDF Intensified Bioreactor Cell Culture Runs



(A) Cell growth and viability data. (B) Total LV production (TU). (C) Fold increase of TU/L bioreactor, TU/cell, and VCD at induction).

Advantages of TFDF Intensification for Clinical Trials

The performance achieved with the 10 L TFDF-intensified cell culture process confirmed that we can meet the LV demand in a smaller bioreactor scale to conduct a typical Phase II/III CAR-T clinical trial with 100 patients recruited. In comparison, a 172 L batch cell culture process, using a 200 L bioreactor vessel, would be needed to produce a similar amount of viral vector. At the 200 L scale, the estimated footprint for the 200 L bioreactor vessel, the required 50 L N-1 and preceding seed train vessels, and the harvest tote would be 200 square feet. A ≥4X reduction in the manufacturing footprint can be expected when running a 4-day TFDF-based perfusion production scenario at 1 VVD, and the total media required is 50 L compared to a total of 222 L in the batch process.

Subsequently, this process results in >4-fold reduction in the required media and nuclease costs through the N-stage production. To put simply, a 200 L scale batch bioreactor would be required to produce 100 doses of CAR-T versus a 10 L TFDF-intensified bioreactor, which would simplify the process as well as reduce the manufacturing footprint and the associated costs (media and nuclease).

Conclusion

The purpose of this study was to showcase the advantages of the TFDF perfusion-based intensification of LV production with stable cell lines compared to a batch process, as well as to highlight its ability to simplify scale-up in clinical manufacturing scenarios. Overall, the data support using TFDF technology to intensify LV production to resolve large-scale manufacturing drawbacks as it can increase LV production >10-fold. This eliminates the need for long and complex seed trains for large-scale production and the preparation and handling of said large-scale production is reduced to bench scale.

Key benefits of TFDF intensification for stable cell line lentiviral production in manufacturing:

- Simplify process steps: TFDF-intensified processes increase LV production >10X and, as a result, can reduce production scale by ~20X when compared to a 200 L batch process.
- Reduce manufacturing footprint: Compared to a 200 L batch manufacturing scenario, the footprint is reduced by ≥4X with a 10 L TFDF-intensified bioreactor.
- Decrease bioreactor volume, media used, and cost: For a 200 L batch manufacturing scenario, the required media volume is reduced by >4-fold, along with the associated cost for media and nuclease when comparing TFDF intensification versus the traditional batch process.

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