CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING & ANALYTICS:
TODAY'S KEY TOOLS AND INNOVATION
REQUIREMENTS TO MEET FUTURE DEMAND

SPOTLIGHT

EXPERT INSIGHT

Lentiviral vector manufacturing process enhancement utilizing TFDF™ technology

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Oxford Biomedica (OXB) is a leading gene and cell therapy company that focusses on Lentiviral Vector (LV) technology innovation, with over 22 years of experience in process development and manufacturing. To address increased LV supply demand, OXB transitioned a GMP LV cell culture manufacturing platform from a cell factory-based adherent cell process to a more scalable, serum-free suspension process performed in single use bioreactors, scaled-up to 200 L in volume at the current time. The relative sensitivity of lentiviral vectors to environmental pH, salt concentration and shear stress during vector harvest and downstream processing continues to present a challenge for the development of efficient manufacturing processes [1]. This work evaluates the TFDF™ (Tangential Flow Depth Filtration) technology developed by Repligen Corporation (Repligen) for the harvest of lentiviral vectors from cell culture supernatant from the bioreactor. The TFDF™ technology effectively separated cells and cell debris from vector particles. Harvest yields typically exceeded 90% with flux rates between 700–750 liters/m^2/hour (LMH) at both 5 and 50 L scales. The tangential mode of TFDF™ was found to be sufficiently gentle on the cells to support multiple harvests, opening the possibility of greatly increasing LV vector production in a perfusion mode.

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THE CHALLENGES OF SCALING UP LENTIVIRAL VECTOR MANUFACTURING

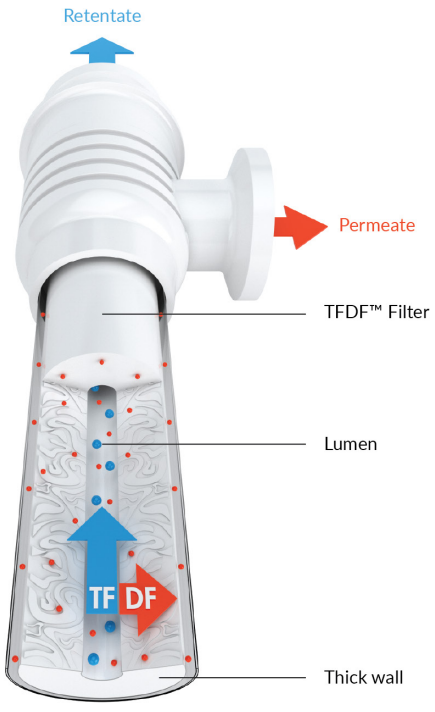
Gene therapy utilizing lentiviral vectors to introduce therapeutic transgenes into the cells of patients represents a unique treatment option for an ever increasing range of genetic and acquired diseases. Key clinical successes, an increase in clinical trial activity in the space, and the approval of lentiviral vector based Advanced Therapy Medicinal Products collectively result in a dramatic increase in global demand for manufacturing capability [2]. However, development of a manufacturing process capable of generating lentiviral products with suitable quality attributes and at the capacity and cost of goods required to provide security of GMP supply remains a challenge across the biotechnology industry. OXB has been a pioneer in the development of products based on lentiviral vectors and has developed a commercial lentiviral manufacturing platform using transient transfection of mammalian cells grown in a serum-free suspension culture. During production, mature vector particles are secreted into the supernatant requiring physical separation of the vector from production cells and cell debris for vector harvest. This initial clarification step is typically performed via normal flow filtration methodologies utilizing filters appropriately sized to enable efficient transmission of vector whilst retaining cells and cell debris. Depth filtration based processes have shown variable recovery and can compromise product quality due to exposure of either the cells or the vector to excessive hydrodynamic stresses [3,4].

TANGENTIAL FLOW DEPTH FILTRATION: A NEW TECHNOLOGY FOR THE ISOLATION OF LENTIVIRAL VECTORS

Repligen recently developed a novel filtration technology, tangential flow depth filtration (TFDF™) based upon passing a cell culture feed stream through a tubular depth filter *in tangential mode* (Figure 1). Cell culture feed travels through the lumen of the tube where retentate returns to the bioreactor and permeate passes through the depth filter wall. The setup of the system resembles that of a hollow fiber but the TFDF™ filter is highly distinct from a hollow fiber. Whilst a hollow fiber measures 0.075 mm to 0.2 mm wall thickness, 0.5 mm to 1 mm lumen diameter, and is typically constructed of mPES, with an anisotropic structure, the TFDF™ filter has 5.0 mm wall thickness, 4.6 mm lumen diameter, and is constructed of polypropylene/polyethylene terephthalate with an isotropic structure (Figure 2). Importantly, the 2–5 µm effective average pore rating of a TFDF™ filter indicates a potential to pass vial vector particles (typically 20–200 nm in diameter) into the permeate whilst retaining the larger

FIGURE 1

TFDF™ tubular depth filter.



Cell culture feed travels through the lumen with tangential flow against the depth filter wall. Retentate (blue) returns to the cell culture feed stock while permeate travels to a permeate reservoir.
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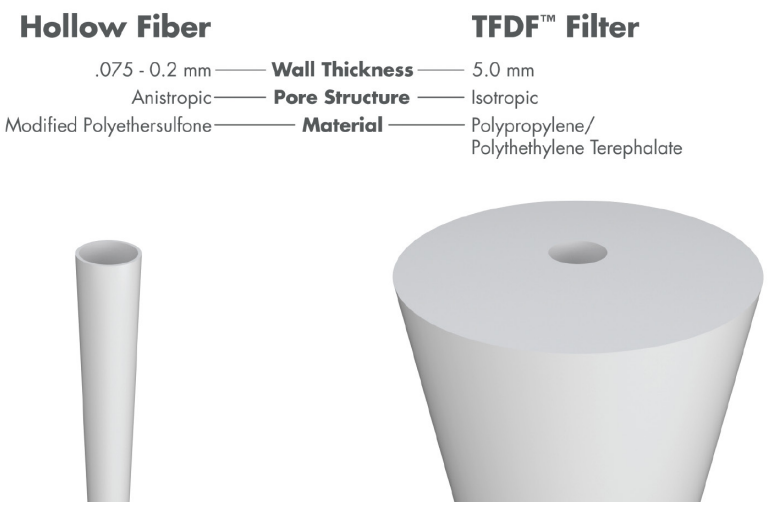
FIGURE 2

Comparison of a hollow fibre filter and TFDF™ tubular depth filter.



cells and cell debris. This study assessed the potential of TFDF™ to separate lentiviral vectors from cells and cell debris during vector production in batch and perfusion modes in bioreactor volumes ranging from 5 to 50 L.

ATTRIBUTES OF THE TFDF™ TECHNOLOGY

The TFDF™ technology leverages aspects of both tangential flow (TF) and depth filtration (DF). Tangential flow through tubular depth filter supports high densities while the structure and capacity of the depth filter enable high product transmission (Figure 3). The combined technology enables cell separation operations of high cell density samples with high flux and high recovery. The filter units scale by increasing both the length of the tube and the number of filter tubes per module (Figure 4).

TANGENTIAL FLOW DEPTH FILTRATION (TFDF™) SYSTEM

The TFDF™ technology comprises hardware, software and ProConnex® TFDF™ flow path components. Both the hardware/software systems and the ProConnex® flow paths have been designed to support bioreactor volumes ranging from 1 to 2000 L (Figure 4). The ProConnex® TFDF™ flow paths are closed, single use, gamma-irradiated and supplied with integrated TFDF™ filter and pressure sensors (feed, retentate and permeate). Genderless AseptiQuik® connectors provide connectivity from the ProConnex® TFDF™ flow path to the bioreactor, additional flow paths and reservoirs. A non-invasive, clamp-on ultrasonic retentate flow meter measures the flow delivered by a magnetic levitating pump from the bioreactor to the filter.

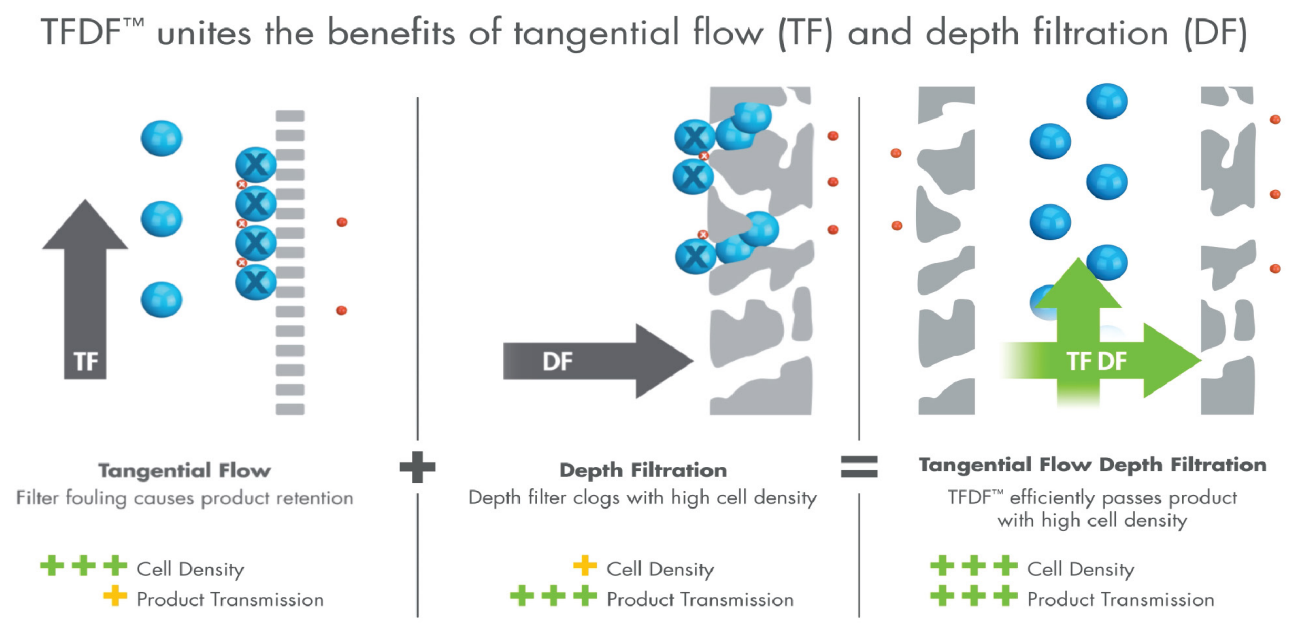
KROSFLO® TFDF™ EVALUATION METHODOLOGY

Experiment #1: harvest of HIV-GFP in batch & perfusion modes

In order to assess the potential use of TFDF™ technology for the isolation of lentiviral vectors during manufacture in serum-free suspension culture, initial studies were performed using a Human Immunodeficiency Virus (HIV-1) vector expressing the Green

FIGURE 3

Tangential flow directs the majority of cells and cell debris over rather than through the filter.



The unique structure of the depth filter enables high product transmission. Operation of a depth filter in tangential mode synergistically enables high yield and flux with high cell density samples.

Fluorescent Protein (GFP) reporter transgene and pseudotyped with the vesicular stomatitis virus G protein (VSV-G). Vector was produced at small-scale in a glass 5 L stirred tank bioreactor (STR) via transient transfection of mammalian cells using lentiviral expression plasmids complexed to a lipid based transfection reagent. To enable harvest of vector via the KrosFlo® TFDF™ System, a ProConnex® TFDF™ flow path with a filter surface area of 55 cm^2 was connected to two *in situ* bioreactor dip-tubes using AseptiQuik® G connectors such that the bioreactor feed and retentate dip-tubes were coupled to their respective lines on the ProConnex® TFDF™ flow path. In a fashion distinct from a hollow fiber tangential flow filtration (TFF) process, the KrosFlo® TFDF™ System applies a pump to the permeate line and during vector harvest the flux for the permeate line was set at 700–750 LMH, a recommended starting point for initial process studies. Following harvest of vector containing supernatant, production cells retained within the bioreactor were re-suspended in fresh media and cultured for an additional period in order to assess the potential for performing multiple vector harvests from a single transient production process. A second vector harvest was performed using the same TFDF™ filter used for initial harvest. In order to assess the efficiency of the TFDF™ harvest process and the impact of TFDF™ harvest on lentiviral functionality, samples of vector containing supernatant were removed from the bioreactor immediately prior to each harvest and also from harvest material collected via the TFDF™ membrane. Functional vector titer in all samples was determined following transduction of adherently grown Human Embryonic Kidney (HEK) 293T cells with transduced (GFP positive) cells quantified using flow cytometry. Briefly, 72 h after viral transduction, treated cells were detached from the assay plate and analyzed for GFP

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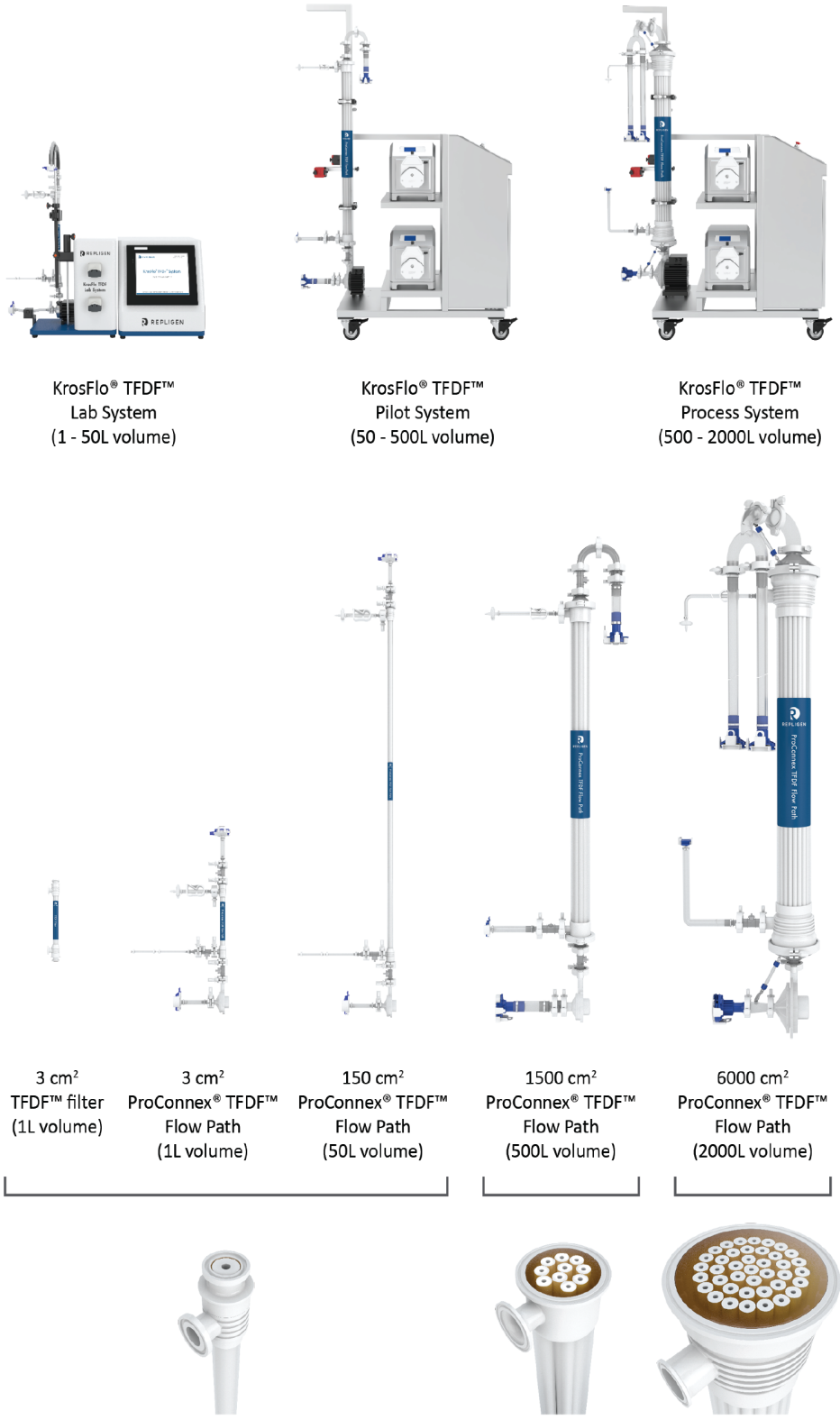
FIGURE 4

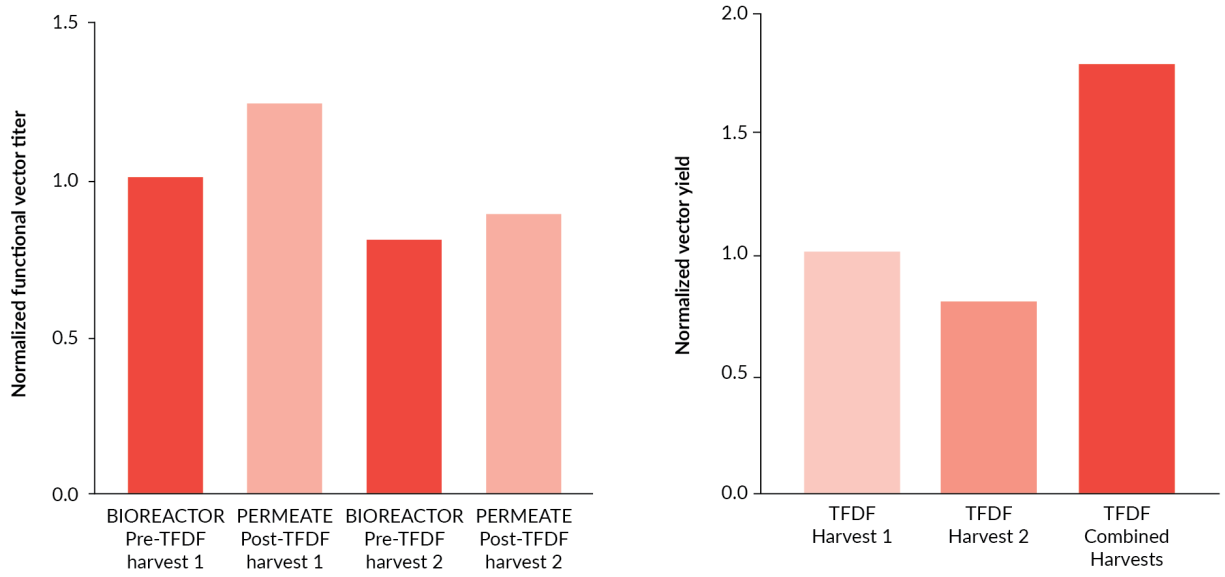
KrosFlo® TFDF™ Systems and ProConnex® TFDF™ flow paths scale from 1 to 2000 L.



The additional flow paths with 55 cm² and 450 cm² surface area are not shown here.

FIGURE 5

(A) Efficient recovery of HIV-GFP lentiviral vector through the TFDF™ membrane. (B) Improved process yields are achieved following multiple TFDF™ harvests.



(A) HIV-GFP vector was produced in a single 5 L STR and harvested from the bioreactor using the TFDF™ system. Following an initial harvest (Harvest 1), cells within the bioreactor were re suspended in fresh media and vector production continued for several hours until a second vector harvest was performed (Harvest 2) using the same TFDF™ system. HIV-GFP vector titers were determined in the bioreactor immediately prior to each harvest and in the TFDF™ permeate following each harvest. All data has been normalized relative to the initial titer in the bioreactor.
(B) Combination of HIV-GFP yields from Harvest 1 and Harvest 2 material resulted in an 80% increase in overall process yield compared to a standard single harvest approach. All values are normalized relative to TFDF™ harvest 1.

fluorescence using a BD FACSVerse™ flow cytometer in conjunction with BD FACSuite™ software. Size and fluorescence data was collected for 10,000 individual events per sample and all samples were analyzed in duplicate. Appropriate analytical gating was utilized to identify the percentage of HEK293T cells that exceeded a fluorescence threshold determined by the background fluorescence measured in non-transduced cells. Functional vector titer was calculated assuming a single transducing unit per GFP positive cell.

Experiment #2: harvest of therapeutic lentiviral vectors

Whilst studies performed utilizing vectors expressing reporter genes such as GFP can be hugely informative for process development, the gene of interest encoded within the lentiviral vector construct can significantly impact both upstream and downstream process performance. Consequently, it is essential that any process development activities are assessed in the context of a directly relevant vector construct. In order to assess the performance of the KrosFlo® TFDF™ System for the harvest of clinically relevant lentiviral vectors, two 5 L STRs were utilized for the production of an Equine Immune Anaemia Virus (EIAV) based lentiviral vector expressing a therapeutic transgene pseudotyped with VSV-G. In one bioreactor, a single vector harvest was performed utilizing the KrosFlo® TFDF™ System operating with an applied permeate flux of 750 LMH. In the second bioreactor, vector was harvested utilizing a commercially available depth filter commonly used for clarification of material derived from mammalian cells grown in suspension culture. Samples were removed from both bioreactors prior to harvest and also from the respective clarified harvest material.

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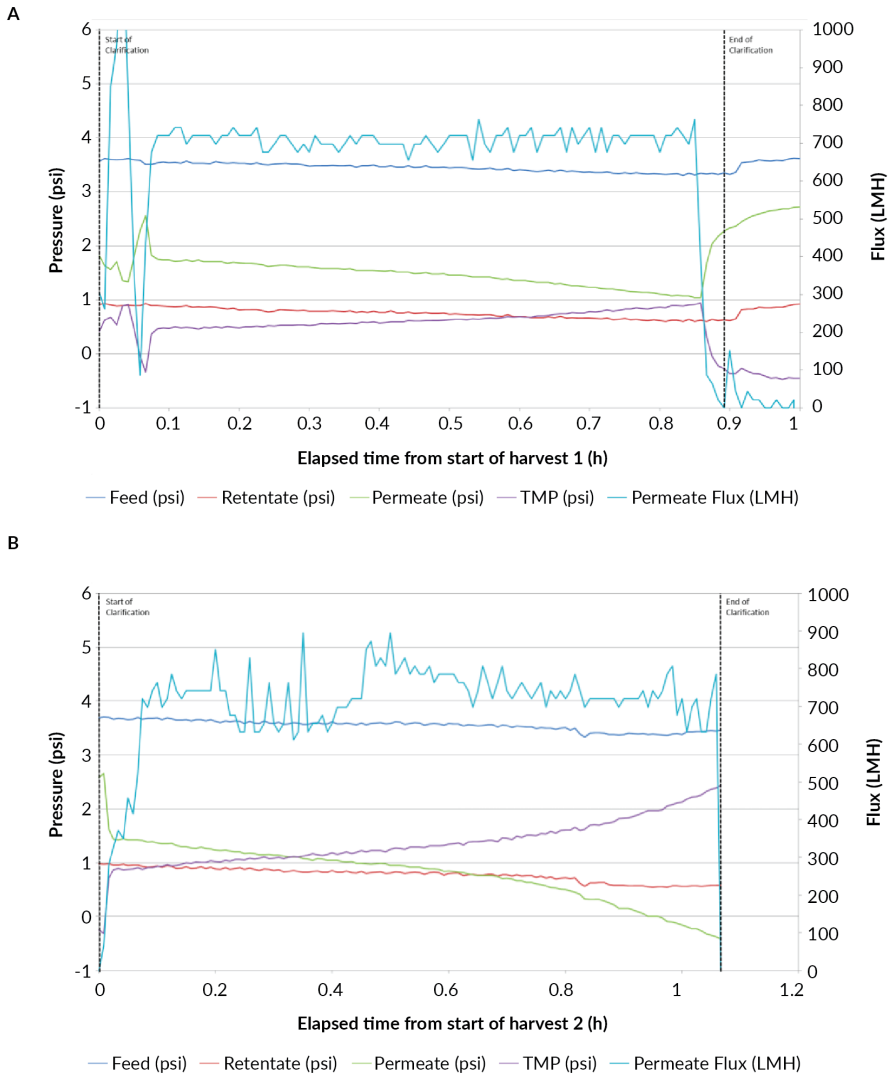
FIGURE 6

(A) TFDF™ Flux and pressure profiles during harvest 1. (B) TFDF™ Flux and pressure profiles during harvest 2.



(A) Flux, feed pressure and retentate pressure remained relatively constant. TMP increased with a slight positive slope and permeate pressure decreased as the inverse of TMP.
(B) Flux, feed pressure and retentate pressure remained relatively constant. TMP increased by 1.5 psi with no impact on flux and permeate pressure decreased as the inverse of TMP.

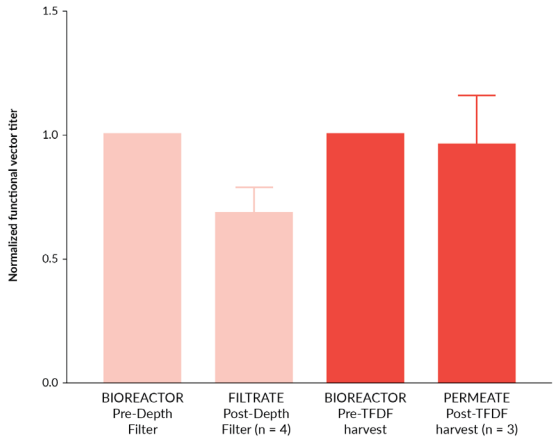
Functional vector titer in all samples was determined following transduction of adherently grown HEK 293T cells with transduced cells identified by immunostaining and subsequent quantification using flow cytometry. Titer determination was performed as described above but transduced cells were identified by flow cytometry following treatment with a fluorescently labelled antibody conjugate targeting the therapeutic protein.

Experiment #3: increased process scale from 5 to 50 L

To evaluate the performance of the KrosFlo® TFDF™ System at a scale more applicable for the clinical manufacture of lentiviral vectors, production and clarification of an EIAV vector incorporating a therapeutic transgene was performed following transient transfection of mammalian cells in a 50 L Single

FIGURE 7

Efficient recovery of a therapeutic EIAV lentiviral vector through the TFDF™ membrane.



EIAV vector expressing a therapeutic transgene was produced in 5 L STRs and vector was harvested from the bioreactor using a standard depth filter approach (n = 4 STRs) or using TFDF™ technology (n = 3 STRs). EIAV vector titer was measured in all bioreactors immediately prior to vector harvest and in the harvested material. Results were used to determine vector recovery during the harvest process. All data is normalized to the titer in the associated production bioreactor and error bars, where present, represent mean ± one standard deviation.

Use Bioreactor (SUB). All vector production process operations were scaled in accordance with accepted engineering principles and in order to accommodate the larger process volume a ProConnex® TFDF™ flow path with a filter surface area of 450 cm^2 was utilized at a permeate flux of 750 LMH for vector harvest. Samples were removed from the bioreactor prior to clarification and also from harvest material collected immediately following clarification through the TFDF™ membrane. Functional vector titer in all samples was determined as described above.

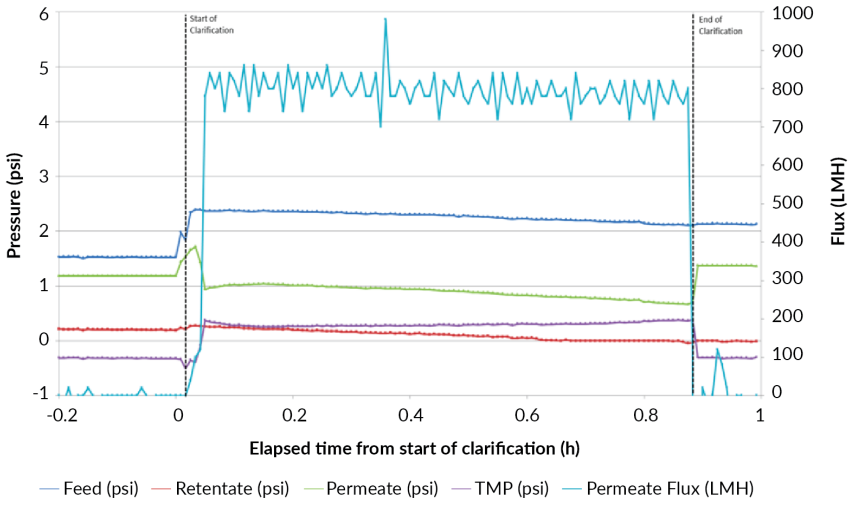
TANGENTIAL FLOW DEPTH FILTRATION: A NEW MODALITY FOR MANUFACTURING LENTIVIRAL VECTORS

This study assessed the feasibility of the TFDF™ technology to harvest lentiviral vector particles in both batch and perfusion modes from serum-free, suspension cell culture. Performance of the TFDF™ technology was compared to a depth filtration method commonly used for clarification of viral supernatant. Experiments were scaled from 5 to 50 L.

Initial evaluation of the TFDF™ technology demonstrated clear separation of HIV-GFP

FIGURE 8

Flux and pressure profiles from TFDF™ system during harvest of a therapeutic lentiviral vector.



During the run, all pressure values were maintained within ±1 psi and a steady permeate flux was achieved.

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lentiviral particles from production cells and cell debris during the harvest process. Analysis of the functional titer in Experiment 1 indicated successful recovery of vector through the TFDF™ membrane at the first harvest and also following a second harvest (Figure 5A). To calculate vector recovery, samples were removed from the bioreactor immediately prior to TFDF™ mediated harvest and from the collected permeate at each harvest time point. For both harvests, the functional titer determined in the bioreactor and associated permeate were similar indicating that there was no measurable loss of vector during TFDF™ mediated harvest. In this initial proof of concept study, the measured functional titers in the TFDF™ permeate samples were marginally higher than those measured in the bioreactor prior to each harvest (Figure 5A). Vector harvest utilizing TFDF™ technology is not associated with concentration of the harvest material and this observation may be attributed to the inherent assay variability associated with titer determination for lentiviral vectors [5]. Importantly, the data demonstrates that the TFDF™ technology is appropriate for use with shear sensitive lentiviral vectors as no loss of vector functionality was observed across the harvest process.

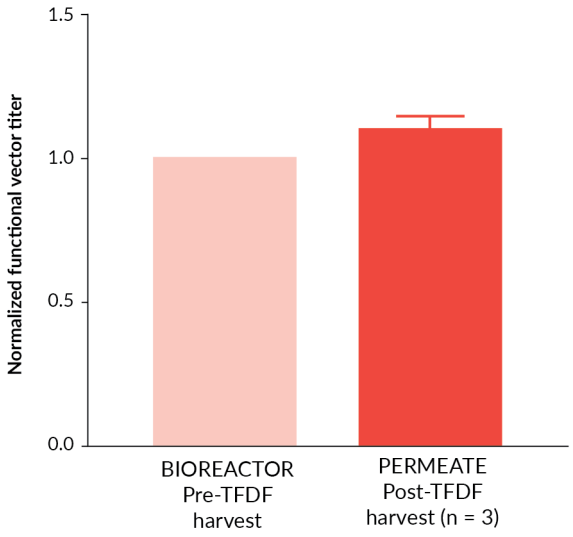
The potential benefits of TFDF™ technology for increasing overall lentiviral vector process yields was demonstrated by the ability to perform multiple vector harvests from a single transient transfection process. Although the observed vector titer in harvest 2 samples was lower than that observed in harvest 1 samples (Figure 5A), pooling of TFDF™ permeate material from both harvests resulted in an increase in overall process yield of approximately 80% compared to the single harvest process (Figure 5B). Multiple harvest steps are not feasible when primary vector clarification is performed using standard depth filtration approaches since the filters result in trapping of the cells outside of the production environment.

Harvest via the TFDF™ system was executed at a relatively high flux of 750 LMH with little evidence of membrane fouling. The flux profile as a function of time indicates relatively minor pressure changes in the feed, retentate, permeate and transmembrane pressures (TMP) over the course of the one hour unit operation (Figure 6A). The only pressure increase of note was a rise of TMP from 0.5 psi at the start of harvest to approximately 1 psi after 45 minutes. Operation at a flux of 750 LMH with a TMP of less than 1 psi differentiates TFDF™ from traditional TFF and depth filtration technologies that operate at significantly higher TMP. The design of the TFDF™ filter itself in combination with a tangential mode that directs the majority of cells and cell debris to be retained within the fiber lumen rather than through the filter is most likely responsible for achieving the observed high flux with low TMP values.

A similar flux profile was generated during harvest 2 using the same TFDF™ filter as harvest 1 (Figure 6B). Flux was again maintained at 750 LMH for approximately 45 minutes. Feed and retentate pressures remained stable

FIGURE 9

Efficient recovery of a therapeutic EIAV lentiviral vector through TFDF™ membrane at 50 L scale.



EIAV vector expressing a therapeutic transgene was produced in 50 L STRs (n = 3) and vector was harvested from the bioreactors using appropriately scaled TFDF™ membranes. EIAV vector titre was measured in all bioreactors immediately prior to vector harvest and in the TFDF™ permeate. Results were used to determine vector recovery during the harvest process. All data is normalised to the titre in the associated production bioreactor and error bars, where present, represent mean ± one standard deviation.

with less than ±1 psi variation. TMP started at approximately 1 psi and increased by only 1.5 psi to a final value of 2.5 psi, indicating that while some cells and cell debris restricted flow through the membrane, the amount did not impact flux.

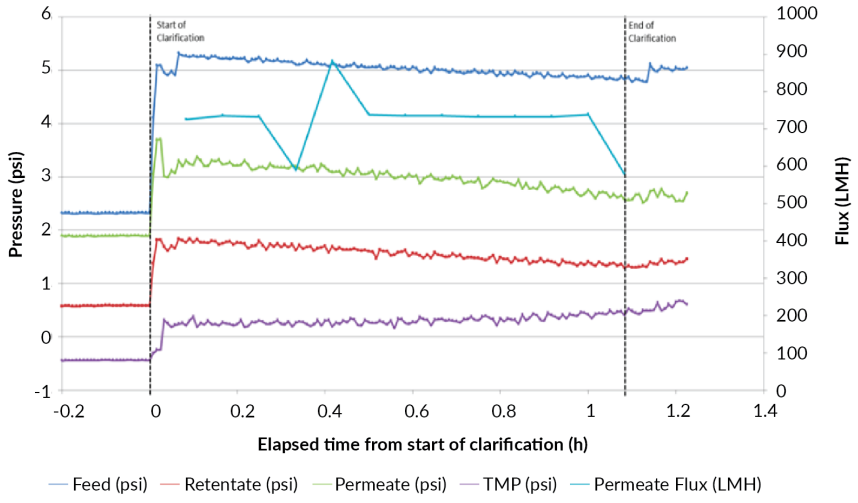
In order to extend the TFDF™ evaluation to include a clinically relevant vector, lentiviral vector harvest utilizing the TFDF™ system was repeated utilizing an EIAV based lentiviral system incorporating a therapeutic transgene. In three independent studies, vector was produced in 5 L STRs and harvested utilizing TFDF™ technology (Figure 7). Similar to results utilizing the HIV GFP vector, efficient transmission of the EIAV vector across the TFDF™ membrane was observed with an average process recovery of approximately 95%. The flux and pressure profiles for the LV vector harvest were similar to those of HIV-GFP (Figure 8). Flux was held constant at 750 LMH and all pressure varied by less than ±1 psi. Similar to runs with HIV-GFP, TMP started at approximately 0.5 psi and progressed with a slight positive slope. The pressure curves illustrate robust filter performance without filter fouling, enabling completion of the harvest unit operation in less than one hour. In contrast to vector harvest utilizing TFDF™ membranes, comparable studies utilizing a standard depth filtration approach for vector harvest in four independent STRs resulted in average vector recovery of only 70% (Figure 7).

Overall, these data demonstrate the efficient recovery of lentiviral vectors utilizing TFDF™ technology and application of a multiple harvest approach could potentially be utilized for further increases in process yield

Process scalability represents a critical requirement of manufacturing technologies. Harvest clarification of an EIAV lentiviral vector expressing a therapeutic transgene was therefore scaled ten-fold from 5 to 50 L in a single-use bioreactor. Vector recovery was assessed in three independent bioreactor studies (Figure 9). In all studies, the ProConnex® TFDF™ flow path was scaled appropriately. While operating at a similar flux of 750 LMH (Figure 10), the TFDF™ technology once again effectively separated cells and cell debris from LV vector at a constant flux with completion of the unit operation in approximately one hour. Comparison of vector

FIGURE 10

TFDF™ Flux and pressure profiles during harvest of a LV vector at a 50 L scale.



Flux, feed pressure and retentate pressure remained stable ± 1 psi. TMP increased with a slight positive slope by 0.5 psi and permeate pressure decreased as the inverse of TMP.

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titer in the permeate to that of the bioreactor again indicated efficient transmission of lentiviral vector across the TFDF™ membrane and complete recovery of the EIAV lentiviral vector was observed (Figure 9).

TOWARDS A PERFUSION MODE?

This study evaluated the TFDF™ filtration technology developed by Repligen for separation of cells and cell debris from lentiviral vectors as a means of harvest clarification in both batch and perfusion mode at scales between 5 and 50 L. This study demonstrates the first practical demonstration and application of this approach for the clarification of lentiviral vector material prior to further downstream purification using the effective 2–5 µm pore rating of the TFDF™ filter. The clarification unit operation was completed in less than an hour, enabled by flux rates between 700–750 LMH, with yields typically greater than 90%. Comparable results were found for both HIV and EIAV based lentiviral vectors and for vectors expressing a reporter gene as well as a therapeutically relevant transgene.

Whilst the high process flux and vector yields observed using TFDF™ mediated harvest clarification offer significant improvements over standard clarification approaches, extension of the TFDF™ to support a perfusion mode for lentiviral vector production holds the potential to significantly increase productivity. Using the HIV-GFP vector, use of TFDF™ as a cell retention device for perfusion of vector particles was shown to be successful with inclusion of a second harvest resulting in an overall increase in process yield of approximately 80%.

Setup and operation of the TFDF™ technology was technically simple, facilitated by the integration of pressure sensors, TFDF™ filter, tubing and clamps into a single ProConnex® flow path, requiring less than 30 minutes for consumable installation and filter priming prior to the run. Users with knowledge of hollow fiber or flat sheet methods will find the overall user experience to be familiar.

To conclude, the KrosFlo® TFDF™ system was found to be an effective tool for lentiviral vector harvest clarification. The process is efficient, easy to use, scalable and offers the potential to improve overall vector yields via repeated harvesting from the production vessel.

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AUTHORSHIP & CONFLICT OF INTEREST

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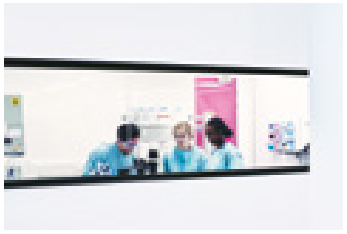

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