Optimizing Lentiviral Manufacturing Economics with TFDF® Technology

Introduction

Gene and cell therapies, with chimeric antigen receptor T cells (CAR-T) representing the most prominent example, hold significant promise towards treating hemoglobinopathies, immunodeficiencies and cancers.[1] Delivery of a gene of interest to a specific target, such as a tumor cell, allows a function to be restored or defect corrected. In the construction of the final gene therapy product, the viral vector component plays an essential role.

Lentiviruses are often chosen as the viral vector due to their effectiveness in transfecting dividing and non-dividing cells and their established safety profile. Additionally, low immunogenicity and toxicity provide opportunities for long-term gene expression in the transduced cell.[2] Thoroughly characterized lentiviruses include the human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2), feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV). Recent regulatory approvals have generated a pathway to therapeutic commercialization for these previously academic or research technologies. As an indicator of the increased utilization, the number of clinical trials using lentiviruses has increased from 200 in 2017 to 640 in October 2020, according to the ClinicalTrials.gov trial registry.[3]

However, supporting large-scale lentivirus manufacturing has presented challenges that impede commercialization.[4] Difficulty generating stable cell lines often necessitates transient transfection of adherent cells. Moreover, traditional filtration methods, such as depth, inflict significant hydrodynamic stress, negatively impacting both product quality and yield.[5,6] Unit operations that produce the viral vector represents a major component to final production cost, driving the development of new and intensified methods to improve patient access through commercial economics. Process intensification plays a key role in maturing manufacturing technologies towards increased productivity and lowered costs (Figure 1), improving the commercial viability of therapeutic candidates and potential market access of commercial therapeutics.

Tangential Flow Depth Filtration (TFDF®) Technology from Repligen Corporation (Repligen) separates cells from media with low shear by combining the beneficial aspects of both tangential flow (TF) and depth filtration (DF) into a single technology. When applied to lentivirus production, TFDF® Technology can address multiple manufacturing challenges through the intensification of bioreactor fermentation and clarification. This application note illustrates a three-step progression from status quo fed-batch bioreactor production with depth filtration to a perfusion-based continuous process with TFDF® Technology. Both process productivity and economics are considered at the liter, batch, dose and annual cost/patient levels.[7] Each progressive step, enabled by the use of the TFDF® Technology, leads to additional increases in process productivity. Each increase in process productivity, in turn, translates to decreased process costs and product costs (Figure 1).

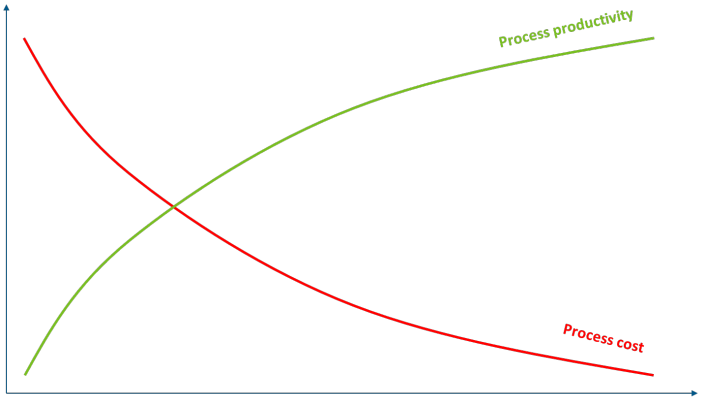

Figure 1. The inverse relationship between process productivity and cost. Increasing process productivity through intensification decreases process costs and, in turn, product cost.

Lentivirus Process Intensification Using TFDF® Technology

TFDF® Technology combines the benefits of tangential flow, which can process high cell density samples, and depth filtration, which can efficiently transmit product, into a single technology-delivering high transmission with high cell density samples. Cell culture feed flows through a tubular depth filter in tangential mode. As the feed travels vertically up through the lumen of the tube, appropriately sized components pass through the depth filter wall as permeate. Components of the feed that complete traveling through the tubular filter are directed back to the bioreactor as retentate (Figure 2). An effective average pore rating of 2 - 5 µm passes viral vector particles, which are typically 20 - 100 nm in diameter, through the TFDF® filter, separating the viral vector from host cells and cell debris.

Figure 2. TFDF® Technology combines the benefits of tangential flow and depth filtration to efficiently pass product using high cell density samples. Lentivirus is recovered in the permeate pool when using a TFDF® filter while production cells are retained in the bioreactor.

A previous publication[7] described the results of a collaborative study by Repligen and Oxford Biomedica (OXB), a leading gene and cell therapy company. In the study, TFDF® Technology recovered approximately 90% of the product while depth filtration only recovered approximately 70% of the product in a single harvest (Figure 3). Therefore, simply substituting depth filtration with TFDF® Technology filtration can improve yield by over 20%. While a 20% increase is significant, further gains are possible due to the fact that TFDF® Technology preserves host cells. Low mechanical stress ensures that retained cells maintain a robust state capable of further production; in a step towards intensification, a single batch of production cells can be leveraged for multiple harvests, each with an average yield of approximately 90%. In the original study, a single batch of production cells was harvested twice to achieve an overall yield improvement of 250% as compared

to a single depth filtration harvest as a proof of concept. With no technical barriers to extending the number of harvests beyond two or progressing to continuous perfusion, the productivity of two fed-batch harvests (from the same batch of production cells) and, finally, continuous production in perfusion mode translates to economic gains (Figure 4).

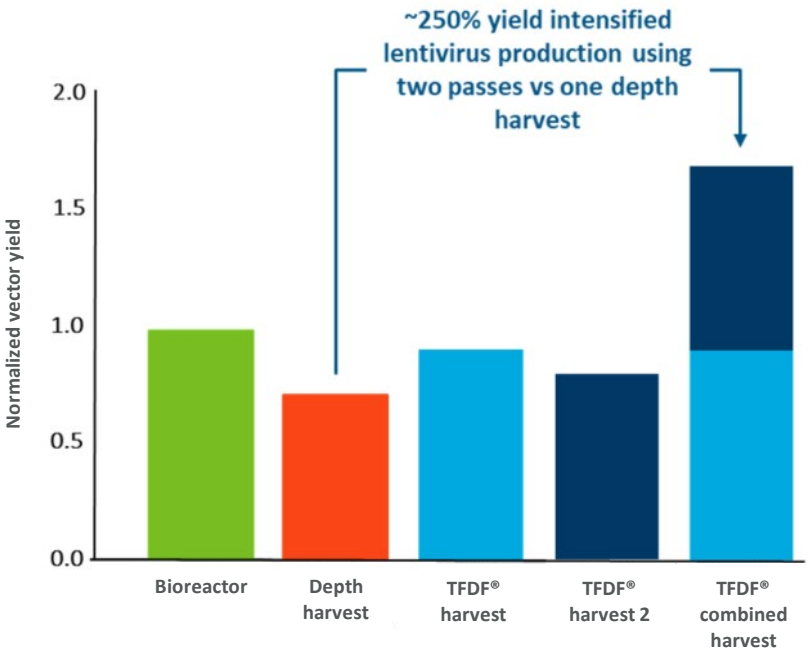

Figure 3. Lentivirus process yield comparison between TFDF® Technology and depth filtration. The TFDF® Technology filter efficiently transmitted virus with ~90% yield in a single harvest while depth filtration achieved ~70% (third bar from left). Low shear cell retention with TFDF® Technology preserved the host cells, enabling intensification through a second harvest without the need for thawing a new cell bank and expansion through a seed train (fourth bar from left). TFDF® Technology achieves an overall 250% yield increase over two harvests in comparison to a single harvest by depth filtration (fifth bar from left).

Figure 4. The progression from depth filtration to perfusion-based continuous processing. Standard depth filtration harvest process (1), TFDF® single harvest (2), TFDF® multi-harvest, the first step towards intensification (3) and intensification with continuous perfusion (4).

Modeling Lentivirus Manufacturing Costs

Data obtained from the Oxford Biomedica study[7] was used to model four different manufacturing scenarios. In this model, lentivirus transduced autologous CD34+ hematopoietic stem cells (HSC), an established approach for the treatment of inherited immunodeficiencies. Using a value of 1x 1010 lentivirus transduction units (TU)/dose, the model included only the major consumables and populated with established process parameters (Table 1).

Table 1. Process parameters and consumable costs used to model four different manufacturing scenarios

	Bioreactor
Process	Transfection with 1 µg of plasmid DNA/1E6 suspension cells
	Virus productivity of 5 TU/cell/day
	25% post-clarification yield
	Post-clarification unit operations: ultrafiltration/diafiltration (UF/DF), chromatography, sterile filtration, formulation/fill.
Consumable costs	GMP grade plasmid DNA: $300,000/g
	Suspension cell culture media: $100/L
	Filters (depth filtration, sterile TFDF®, UF/DF): $5,000/$m^2$
	Chromatography media: $5,000/L

Non-optimized downstream leads to low yields

For each of the four manufacturing processes considered, cell harvest or retention yield was based on the results from the referenced Repligen-Oxford Biomedica publication (Table 2).[7] The two harvest TFDF® Technology process included replenishment of the bioreactor with fresh media following the first harvest. The perfusion process runs harvest continuously, utilizing the TFDF® Technology filter as a cell retention device both before and after transfection.

Table 2. Parameters used to model the economics of four different lentivirus manufacturing processes

	Batch - Depth filtration (1 harvest)	Batch - TFDF® (1 harvest)	Batch - TFDF® (2 harvests)	Perfusion - TFDF® (continuous harvest)
Cell culture mode	Batch	Batch	Batch	Perfusion
Bioreactor seed train (L)	200/500	200/500	200/500	20/50
Production Bioreactor volume (L)	2000	2000	2000	200
Viable cell density (cells/mL)	$2x10^6$	$2x10^6$	$2x10^6$	$2x10^7$
Virus production phase (days)	2	2	3.5	3.5
Filtration technology	Depth	TFDF®	TFDF®	TFDF®
Harvest/retention yield (%)	70	90	90	90

Up to 20-fold More Doses/Harvest Using TFDF® Technology

Process economics were first analyzed by considering the number of doses obtained per bioreactor liter. Using parameters from Table 1, the expected retention yields and the total number of potential viral genomes (VG)/batch were calculated. With a depth filtration process defined as a productivity level of 1, the results indicated potential productivity increases ranging from 1.29 to 22.50-fold. Simple substitution of depth filtration with TFDF® technology, increased productivity by 1.29x (Table 3, Figure 5). The tangential component of TFDF® Technology plays a key role in creating the 1.29-fold increase with a low hold-up volume. Because the feed stream passes over the depth filter continuously, product is not trapped in a dead-end fouled filter and sacrificed. More significant yield increases beyond 1.29-fold are available when the unit operation is intensified and the cells retained by TFDF® Technology are leveraged for additional harvests. Extension beyond a single harvest can be done in multiple modes. In a multi-harvest mode, harvest is started and completed at discrete time points in a fed batch manner. Multi-harvest execution is straightforward as it resembles a standard fed-batch harvest in nearly every aspect. The process itself defines each harvest and product quality analytics may be used to guide the decision on whether to pool or not pool each harvest. Transitioning from a fed-batch mode to perfusion mode truly maximizes the benefits of productive cell retention by TFDF® Technology. Perfusion can increase cell density 10-fold, decreasing bioreactor size 10-fold and increasing overall productivity/L by up to 22.50-fold. Therefore, if depth filtration produces 0.35 doses/L, TFDF® Technology in perfusion mode could produce 7.9 doses/L.

Table 3. Comparative estimation of the number of doses per bioreactor liter produced with depth filtration, TFDF® Technology fed-batch and TFDF® perfusion processes

	Batch - Depth filtration (1 harvest)	Batch - TFDF® (1 harvest)	Batch - TFDF® (2 harvests)	Perfusion - TFDF® (continuous harvest)
Total VG/batch	$7.0x10^{12}$	$9.0x10^{12}$	$15.8x10^{12}$	$15.8x10^{12}$
# Doses/batch	702	903	1581	1581
# Doses/L bioreactor	0.35	0.45	0.79	7.9

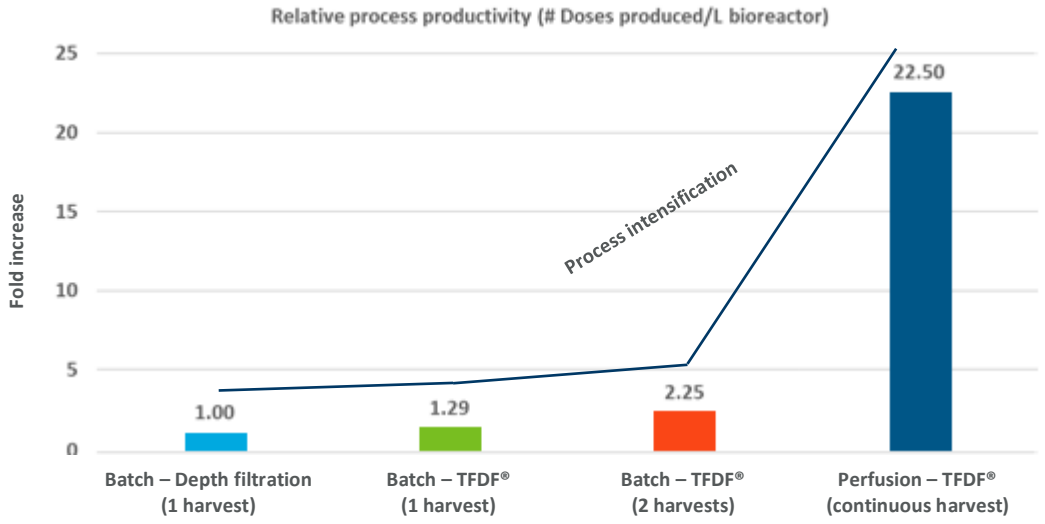

Figure 5. The fold increase of TFDF® Technology applied in three different modes was compared to depth filtration. TFDF® Technology can increase the number of doses produced between 1.29 to 22.5-fold relative to depth filtration. Substitution of depth filtration with TFDF® filtration increases the yield by 1.29-fold. Executing two harvests from the same production cells increases yield 2.25-fold. Conversion to a perfusion mode with a continuous harvest increases the yield 22.5-fold.

Up to 60% consumable cost reduction with TFDF® Technology

Lentiviral process intensification with TFDF® Technology couples increased productivity with consumable cost reduction. Once again using doses/L as a metric, the consumable cost for a TFDF® filtration and depth filtration process was calculated (Table 4, Figure 6).

As a baseline, consumable cost/dose was approximated at $2,500/dose, indicating its significant contribution to overall viral vector cost. Substitution of depth filtration with TFDF® Technology for a single harvest increases doses 1.29-fold/batch and, in turn, decreases consumable cost/dose. Consumable costs with TFDF® Technology drop 21% to $1,946/dose. The two harvest scenarios with a 2.25-fold productivity gain further reduces costs by 45% to $1,367/dose. Lastly, running TFDF® Technology in perfusion mode reduces costs by 60% to $988/dose.

Table 4. Comparative estimation of the number of doses per bioreactor liter produced with depth filtration, TFDF® Technology fed-batch and TFDF® perfusion processes

	Batch - Depth filtration (1 harvest)	Batch - TFDF® (1 harvest)	Batch - TFDF® (2 harvests)	Perfusion - TFDF® (continuous harvest)
# Doses/batch	702	903	1581	1581
Consumable cost/dose ($)	$2,471	$1,946	$1,367	$988
Consumable cost/dose relative depth filtration(%)	100%	79%	55%	40%

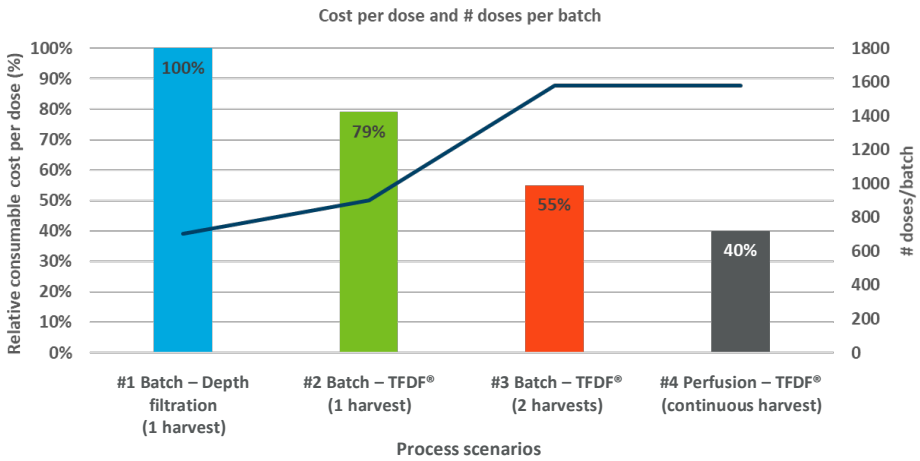

Figure 6. Lentivirus manufacturing cost analysis on the cost of consumables required/dose for each scenario. Consumable costs using TFDF® Technology was 79%, 55% and 40% of the consumable costs required for depth filtration for a one harvest, two harvests and perfusion mode, respectively.

A more encompassing account of consumable costs can be generated by considering total patient demand. The number of patients that requires one dose/year is not always equally divisible by the number of doses produced/batch, creating some dependence of the final value on the exact number of patients; if every dose of a batch is used then cost/patient is lower than if the majority of a batch goes unused. his level of complexity is introduced to the model as scale-up is a tightly controlled process that rarely, if ever, allows for partial batches in response to market demand.

Based on the output of each manufacturing process scenario, the number of batches required to treat 5,000 patients/year was estimated and used to calculate the cost of consumables/year (Table 5, Figure 6). To meet the demand of 5,000 patients/year, depth filtration required eight batches at an average batch cost of $1.73 M and an annual cost of $13.82 M. Clarification with TFDF® Technology using one harvest reduced the number of batches by 2 to 6 at an average batch cost comparable to depth filtration at $1.76 M and a reduced annual cost of $10.54 M. Executing two harvests with TFDF® Technology in multi-harvest mode reduces the number of batches to four. Interestingly, two TFDF® Technology harvests increase the cost/batch moderately to $2.16 M but significantly reduces the annual cost to $8.62 M. Conversion from batch to perfusion, as expected, provides the most favorable economics with a batch cost of $1.56 M and an annual cost of only $6.25 M-. Whether in single harvest batch, two harvest batch or perfusion mode, therefore, TFDF® Technology provides improved overall manufacturing economics that are 76%, 62% and 45% of depth filtration respectively. In this simulated use case, an annual cost reduction of transitioning from a fed-batch process with depth filtration to a perfusion process with TFDF® Technology can reach $7.58 M annually. If the number of patients is increased from 5,000 to 10,000 or even 15,000 patients/year then the economic benefits are multiplied two- and three-fold (Figure 6)

Table 5. Comparative estimation of the cost required to meet the demand/patient/year produced with depth filtration, TFDF® Technology fed-batch and TFDF® perfusion processes

	Batch - Depth filtration (1 harvest)	Batch - TFDF® (1 harvest)	Batch - TFDF® (2 harvests)	Perfusion - TFDF® (continuous harvest)
# Doses/batch	702	903	1581	1581
# Patients/year	5000			
Batches/year	8	6	4	4
Cost /batch ($M)	1.73	1.76	2.16	1.56
Cost ($M)/year	13.82	10.54	8.62	6.25
Relative cost (% of depth filtration)	100%	76%	62%	45%

Conclusions

Gene and cell therapies hold wondrous promise to address unmet medical conditions. More traditional therapeutics such as small molecules, recombinant proteins and monoclonal antibodies typically require years to progress through the discovery and development stage. After the definition of the therapeutic, however, decades of manufacturing development have equipped these traditional therapeutics with economically viable and scalable platforms. Conversely, gene and cell therapy early development can occur rapidly, potentially within month, with an economically viable and scalable manufacturing platform standing as a significant hurdle. TFDF® Technology helps overcome manufacturing yield challenges by increasing productivity/L, streamlining the harvest unit operation and improving overall process economics. Previous work demonstrated the compatibility of HEK293 cells with TFDF® Technology filtration for both a single harvest and multi-harvest mode. Here, we model the extension of TFDF® beyond fed-batch to perfusion and estimate productivity gains/L and improved economics/year. TFDF® Technology improves productivity and economics in all three scenarios considered. The fact that benefits are observed under all three scenarios plays a key role. One may elect to rapidly implement the technology as a single fed-batch harvest unit operation and then progressively build towards perfusion-realizing productivity and economic gains throughout.

References

1. Papanikolaou E. and Bosio A., The promise and the hope of gene therapy. Frontiers in Genome Editing. 2021; 24(3):4.
2. Lana M.G. and Strauss B.E., Production of lentivirus for the establishment of CAR-T cells. Methods Mol Biol. 2020; 2086:61-67.
3. Clinical Trials.gov Database. Search: Clinical Trials Using Lentiviral Vectors.
4. McCarron A. et al., Challenges of up-scaling lentivirus production and process. J. Biotechnol. 2016; 240:23-30.
5. Moss D. Vector purification: issues and challenges with currently available technologies. Cell Gene Ther. Ins. 2019; 5(9): 1125–32.
6. Raghavan B. et al. Optimizing the clarification of industrial scale viral vector culture for gene therapy. Cell Gene Ther. Ins. 2019; 5(9): 1311–22.
7. Williams T. et al., Lentiviral vector manufacturing process enhancement utilizing TFDF™ technology, Cell & Gene Therapy Insights 2020; 6(3), 455–467.