Continuous VHU™ Perfusion for an order of magnitude increase in lentiviral vector production

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Introduction

- Lentiviral vectors (LV) represent a key tool for gene and cell therapy.
- The production of these vectors in sufficient quantities for clinical applications leads the field toward developing suspension processes more amenable to large-scale production.
- The HEK293 cell line employed grows in suspension, thus offering direct scalability, and produces a lentiviral vector in the 10^6 transduction units (TU)/mL range without optimization[1].
- This study describes a LV production strategy using either a transient transfection or a stable inducible producer cell line grown in suspension using a novel perfusion process[2].

Methods

- A 3L bioreactor (BioBLU® 3c) was operated (37°C, pH 7.1, DO 40%, 80rpm) in perfusion mode using a novel cell retention device (VHU) (Figure 1).
- Cells were grown for 6 days with a perfusion rate of 0.5 volume vessel per day (vvd).
- Continuous VHU™ Perfusion increases the cell density up to 80x10^6 c/mL.
- Addition of inducers marked the beginning of the production phase and was considered Day 0. Continuous harvests were performed for up to 4 days post-induction (dpi) (Figure 2).

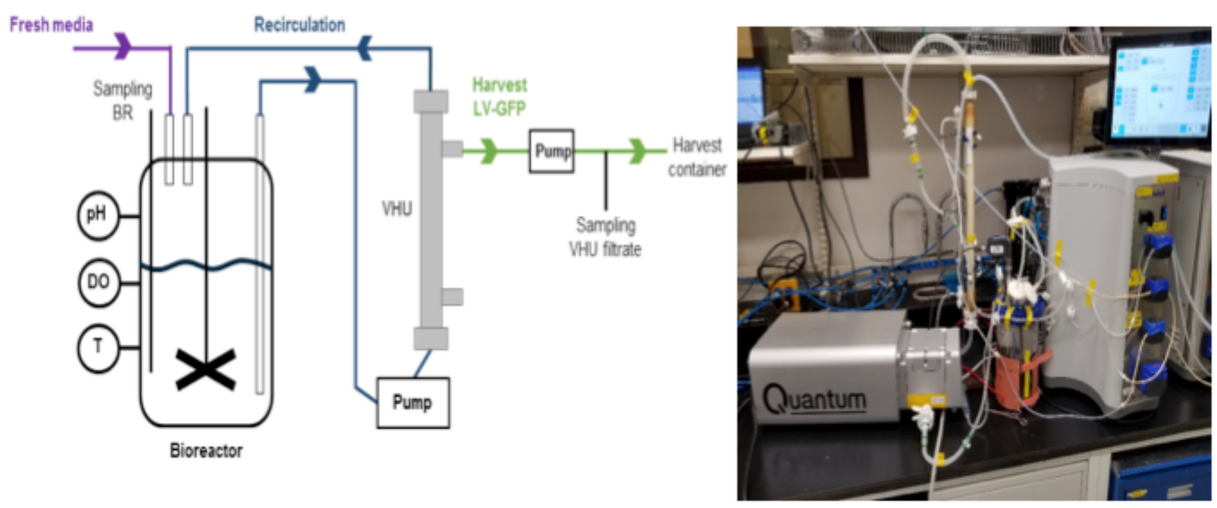

Figure 1 The VHU™ Perfusion Bioreactor.
The integration of Viral Harvest Unit (VHU) (Top) and a low-shear pump (Quantum 600, Watson-Marlow) in TFF mode (Bottom) comprised the bioreactor perfusion system.

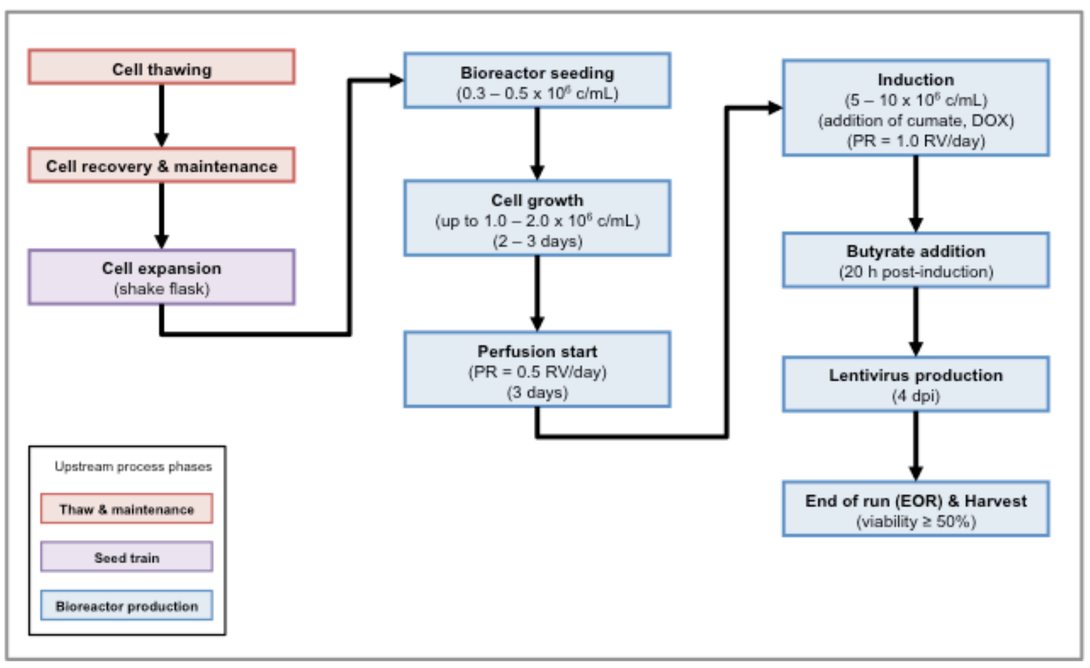

Figure 2. VHU™ Perfusion Process using a Stable Producer Cell Line

Results

- **VHU™ Process Intensification** to increase HEK293 cell density up to 80 million cells/mL and with no cells in the perfusate (Figure 3).

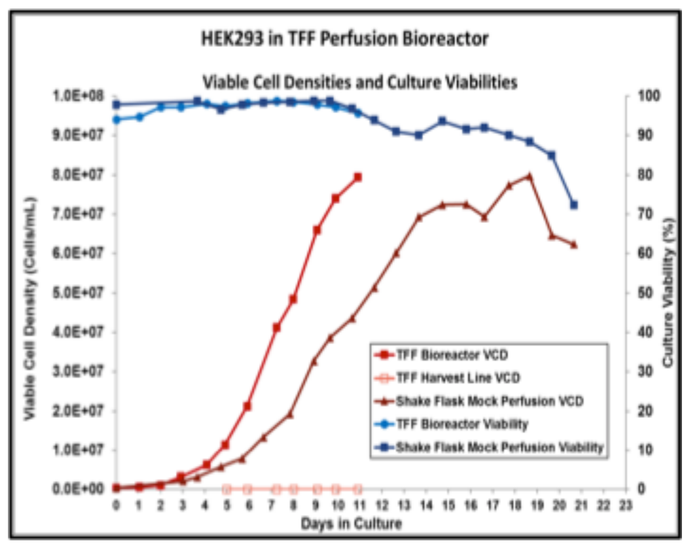

Figure 3. VHU Perfusion compared to shake flask centrifugation. Viable cell density using VHU (red circles) versus shake flask mock perfusion (red triangles)

- Titer and Total Yield were 30-fold higher compared to Batch Mode (Figure 4).

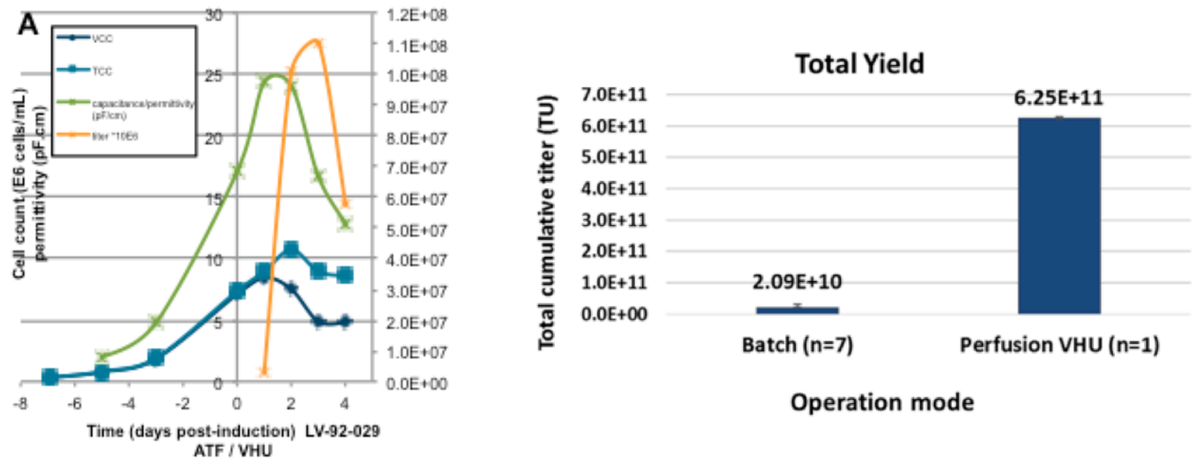

Figure 4. (Left) Growth curve, Capacitance and Titer (GTA) for VHU™; (Right) Total LV titers for Batch vs. Perfusion with VHU™ Filter.

- VHU™ Perfusion provided a clarified stream of <16 NTU compared to <500 NTU for a batch culture (Figure 5 and Table 1).

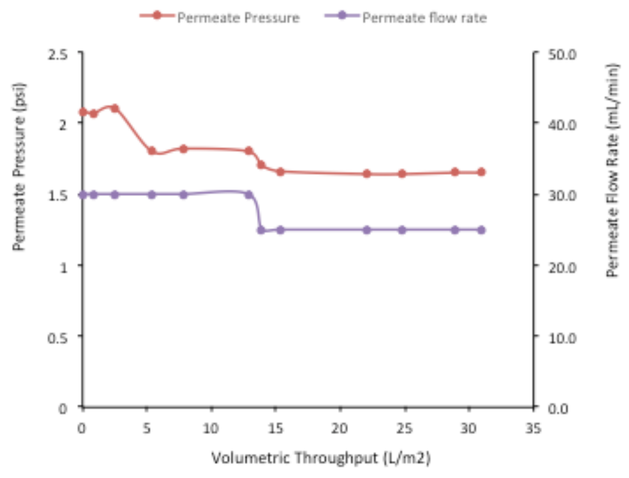

Figure 5. Downstream Processing. LV Harvesting: Feed Flowrate: 3L/min; Permeate: 30mL/min; Clarified volume: 1.86 L; Functional titer: 7.58E+06 TU/mL

Process Mode	Turbidity (NTU)		
	Broth	Post-perfusion device	clarified
Batch	50-90	N/A	Target: ~5
Perfusion VHU	<500	<16	N/A

Table 1. Comparison of turbidity (NTU) between batch and perfusion cell retention devices such as the VHU™

Conclusions

- The lentivirus was continuously harvested using the VHU™ for 4 days post induction.
- The VHU™ offers the potential to eliminate the clarification step.
- Batch vs. Perfusion: Perfusion ~3-times more expensive, 30-fold higher yield.
- Potential for additional higher yields using Process Intensification (80 million c/mL) in either transient transfection or stable producer cell line mode.

REFERENCES
1. Manceur A. et al, Scalable lentiviral vector production using stable HEK293SF producer cell lines. Hum Gene Ther Methods. 2017
2. Cattaneo M. and Spanjaard R. Perfusion Filtration Systems, US Pat. 10,358,626 B2, 2019