Intensified Lentiviral Vector Manufacturing Using KrosFlo® TFDF® Tangential Flow Depth Filtration System

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INSPIRING ADVANCES IN BIOPROCESSING

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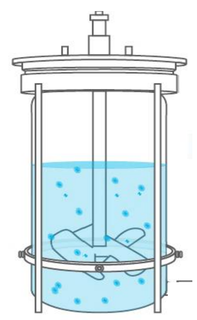
Summary

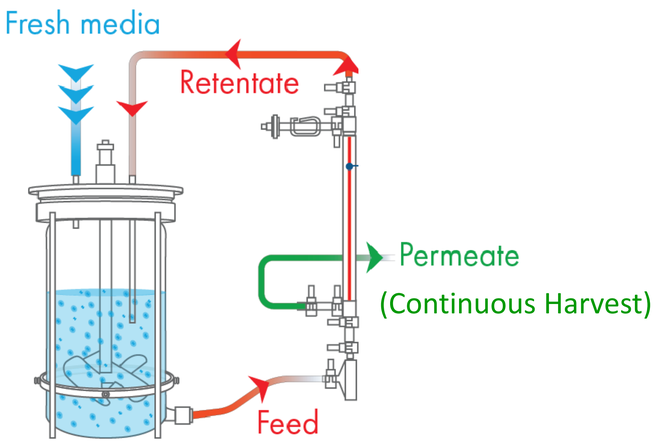
- Cell&Gene therapy promises to cure diseases that currently have limited treatments. Current Lentiviral vector (LVV) manufacturing processes for most of these therapies can serve a limited patient population at low dosage but lack the productivity and yield to supply high dosages to large patient populations. Process intensification and optimization can help overcome this manufacturing challenge
- We used an integrated perfusion platform, the KrosFlo® TFDF® (tangential flow depth filtration) technique, to intensify cell growth and viral vector production
- The perfusion process before transfection enabled a 3 times higher cell density at transfection (~9E6 cells/mL) compared to batch (~3E6 cells/mL)
- Post transfection, perfusion was maintained to limit cell death and permit the continuous harvest of virus particles produced through the 2-5um KrosFlo TFDF pores.
- This intensified strategy, with perfusion cell culture before and after transfection, increased LVV cell specific viral vector productivity (TU/cell) between ~30-fold compared to a regular batch process
- In total, implementing the KrosFlo TFDF-based perfusion led to >80-fold (LVV) total virus yield compared to batch processes

Collectively, the present study paves the way for the development of integrated and continuous viral vector production to meet the global demand and realize the full potential of gene therapy

KrosFlo TFDF-based intensified LVV production



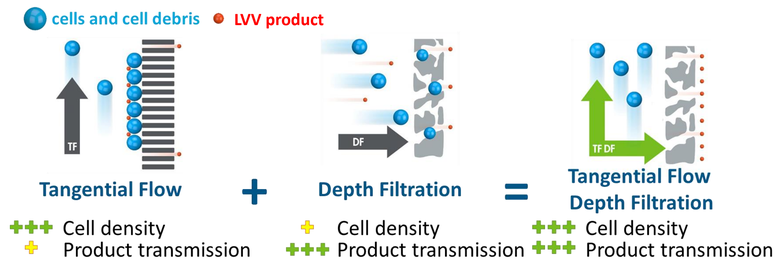
Batch cell culture
3E6 cells/mL VCD at transfection



Krosflo TFDF perfusion-based intensified cell culture
9E6 cells/mL VCD at transfection

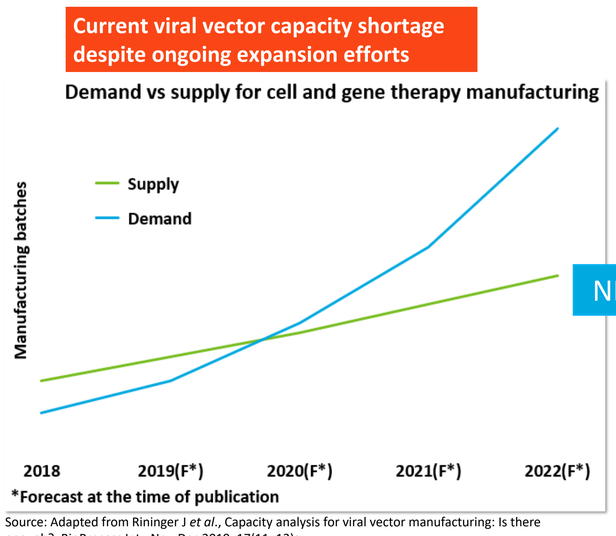
Cell culture conditions for LVV production

- HEK293F cells, Expi293 Expression Medium, Eppendorf BioBlu 3C single-use bioreactor (Eppendorf BioFlo 320 Controller)
- Transfect on Day 3 (Prototype reagent from Polyplus)
- Batch process
  - Seed at 0.4E6 cells/mL & transfect at ~3E6 cells/mL
- Intensified process
  - KrosFlo TFDF (2-5 µm) module (30 cm^2) and lab system connected to bioreactor
  - Seed at 1.1E6 cells/mL & transfect at ~9E6 cells/mL
  - Perfusion started 24 or 48 hours after inoculation at 1 vessel volume per day (vvd)
  - Perfusion post transfection at 1 vvd for continuous harvest into 4 degree C



Increased LVV manufacturing productivity with intensified & integrated processes

Current viral vector capacity shortage despite ongoing expansion efforts



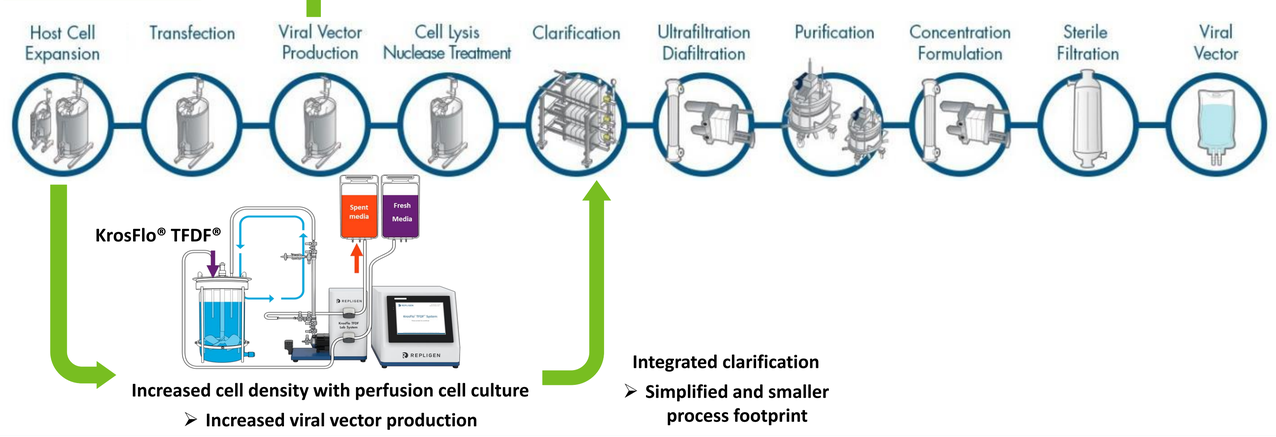
Source: Adapted from Rininger J *et al.*, Capacity analysis for viral vector manufacturing: Is there enough?, BioProcess Int., Nov-Dec 2019, 17(11–12)s

LVV

NEED

Increase productivity

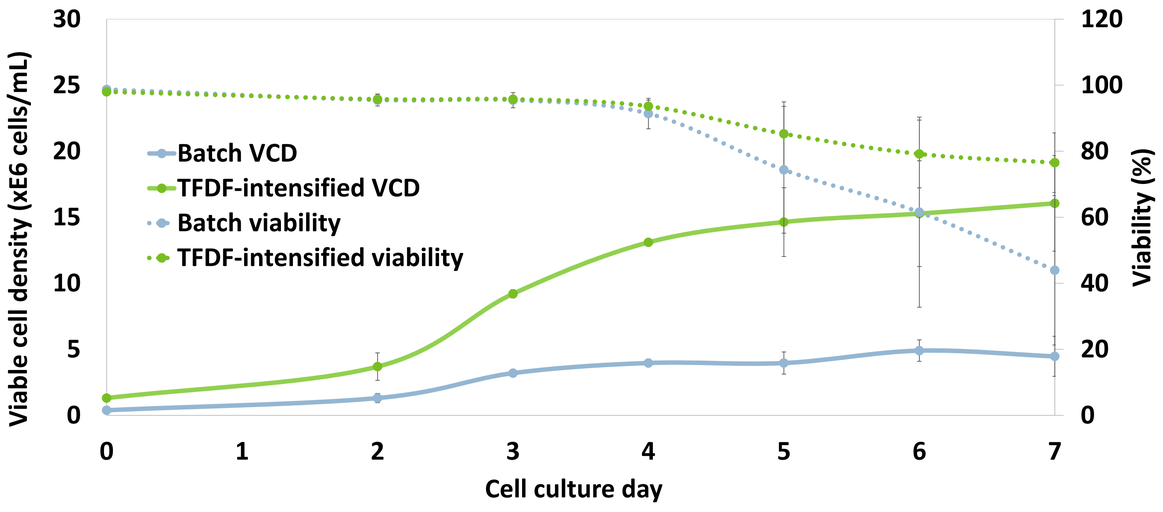
Continuous harvest in perfusion mode
➢ Increased yield (high productivity, long production phase, stabilized virus)



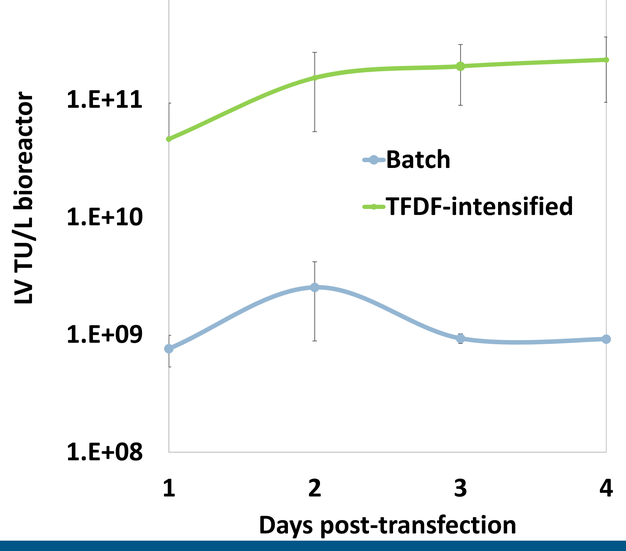
Increased cell density with perfusion cell culture
➢ Increased viral vector production

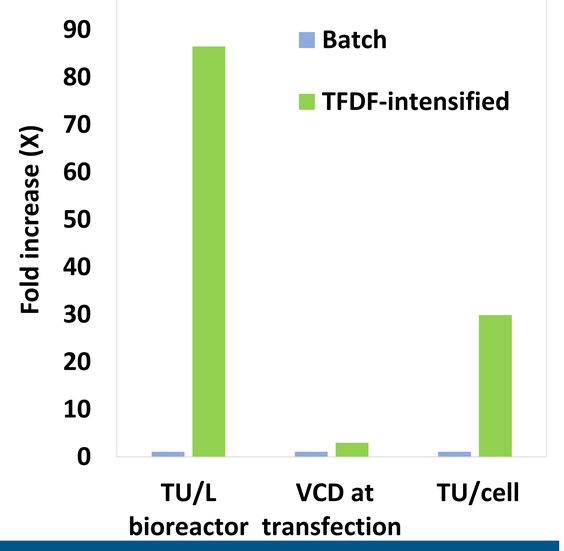
Integrated clarification
➢ Simplified and smaller process footprint

Intensified cell growth for LVV production



Intensified LVV production data





Conclusions

KrosFlo TFDF-based perfusion culture intensification:

- Increased number of cells producing the virus
- No limitation for virus production from nutrient deprivation and/or accumulation of inhibitory metabolites
  - ➢ >80X LVV production
  - ➢ which results from a ~3X increased cell density at transfection
  - ➢ continuous harvest virus stabilization factor into 4 degree C
  - ➢ and close to 30X increased cell specific virus productivity

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