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

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Summary

Significant manufacturing productivity improvements are needed to meet the growing demand for viral vector or gene therapy.

We used an integrated perfusion platform, using 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).

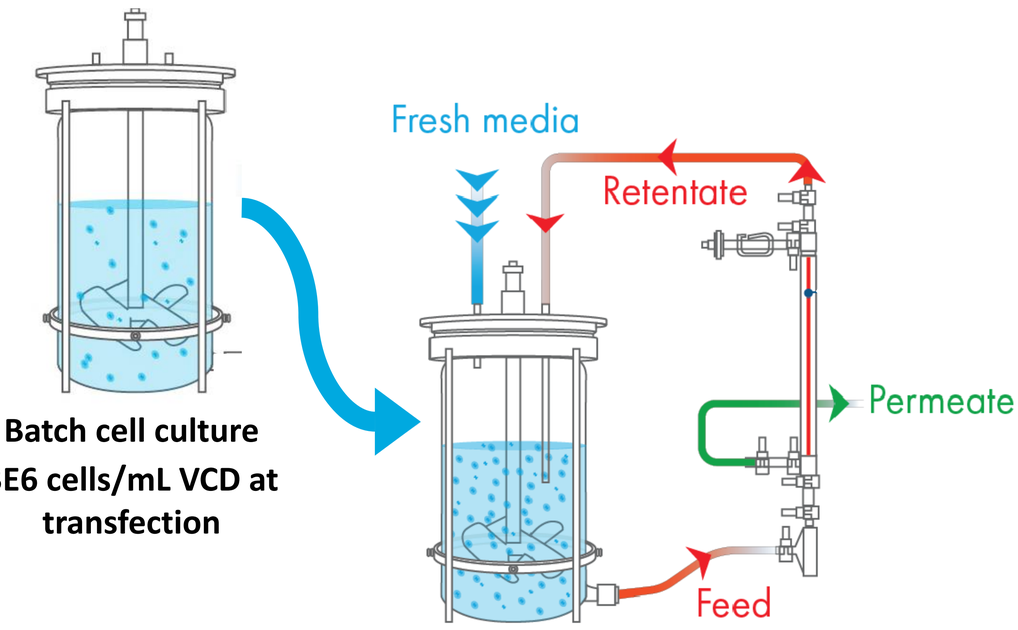
Post transfection, perfusion was maintained to limit cell death and permit the continuous harvest of virus particles produced through the 2-5um KrosFlo TFDF pores.

This intensified strategy, with perfusion cell culture before and after transfection, increased AAV8 and Lentivirus (LV) cell specific viral vector productivity (gc/cell or TU/cell) between ~3 to 30-fold compared to a regular batch process.

In total, implementing the KrosFlo TFDF-based perfusion led to ~10-fold (AAV8) and >80-fold (LV) 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 viral vector production

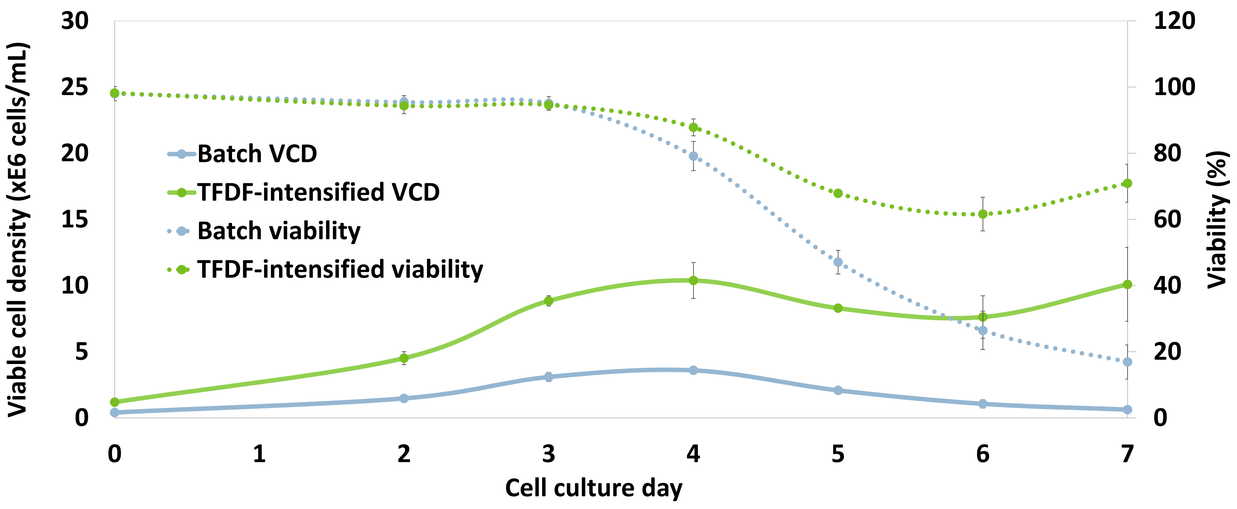


Krosflo TFDF perfusion-based intensified cell culture
9E6 cells/mL VCD at transfection

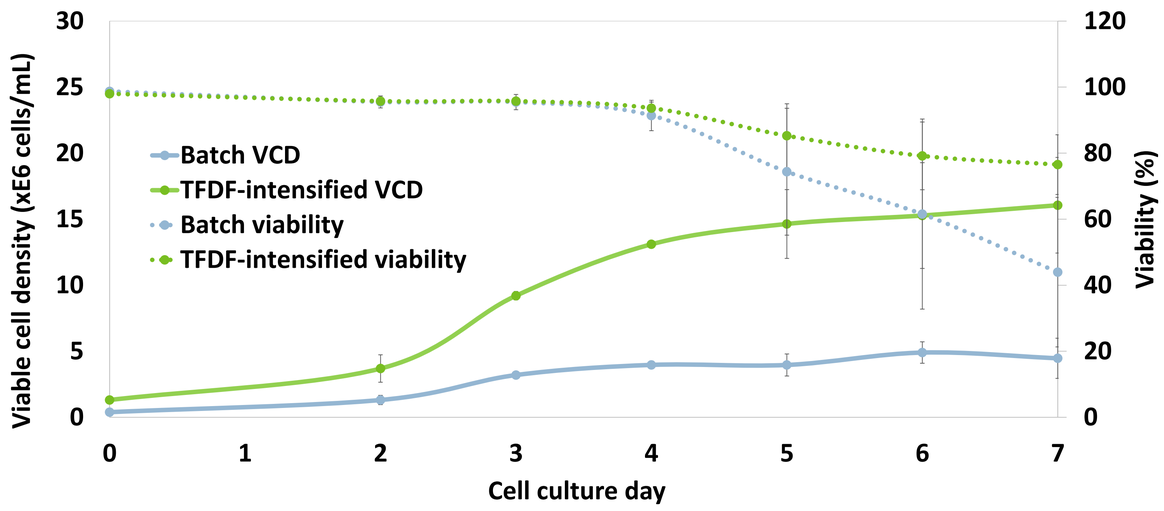
Cell culture conditions for AAV8 and LV production

- HEK293F cells, Expi293 Expression Medium, Eppendorf BioBlu 3C single-use bioreactor (Eppendorf BioFlo 320 Controller)
- Transfect on Day 3 (AAV8: TransIT-VirusGEN AAV Kit ; LV: 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
  - Perfusion started 24 or 48 hours after inoculation at 1 or 1.5 vessel volume per day (vvd) for LV or AAV8 respectively
  - Seed at 1.1E6 cells/mL & transfect at ~9E6 cells/mL
  - Perfusion post transfection at 1.5 (AAV8) or 1 (LV) vvd for continuous harvest (LV: daily harvest stored at 4 degree C)

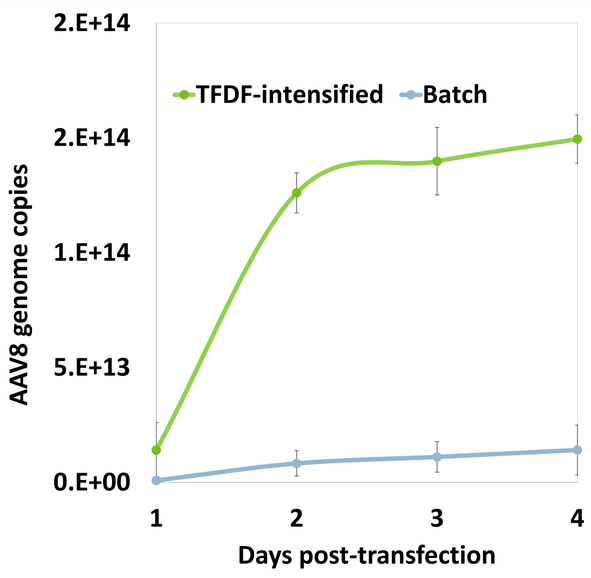
Cell culture data of AAV8 production

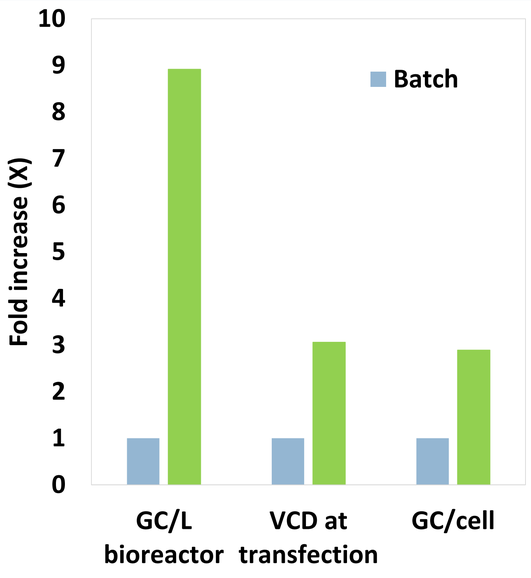


Cell culture data of LV production

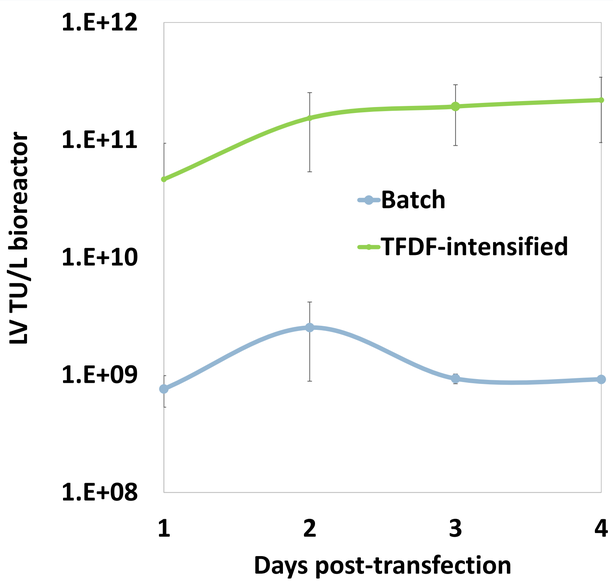


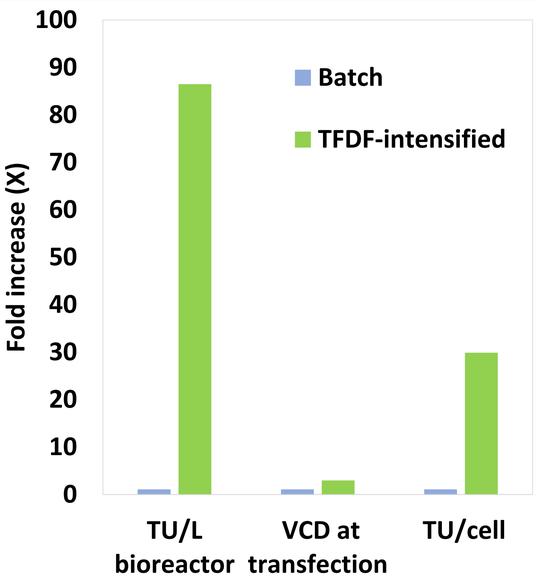
AAV8 production data





LV 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
  - ➢ ~10X AAV8 production which results from a ~3X increased cell density at transfection and a >3X increased cell specific virus productivity
  - ➢ >80X LV production which results from a ~3X increased cell density at transfection and close to 30X increased cell specific virus productivity