Advanced lentivirus production: Insights from cell retention filter-based perfusion process for scalable and cost-efficient manufacturing

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Summary

Tangential flow depth filtration (TFDF®) used for cell retention in perfusion cell culture enables intensified lentiviral vector (LVV) production versus batch processes, significantly enhancing yield and reducing costs. This improvement is driven by a synergistic effect of higher cell density, continuous nutrient feed and waste removal, and cold storage of TFDF-permeate that preserves virus infectivity.

In the first part of the present study, TFDF-based perfusion cell culture runs in a scale-down 250 mL bioreactor were performed to investigate the individual role of these three process parameters to increase LVV production in TFDF-intensified processes.

- **TFDF-based perfusion enabled intensified cell growth to 3X higher viable cell density (12E6 VCD) before transfection compared to a batch control (~4E6 VCD) .**
- **Continuous perfusion post transfection, with the TFDF permeate kept at 37°C, enabled a ~12X increased LVV production in a 2-day production compared to batch control, indicating a 3-fold increase from high cell density, and a ~4-fold increased cell specific productivity from continuous feed and waste removal.**
- **Continuous harvest into cold storage, extending the harvest by an additional day, enabled a 16-fold increase in LVV production over a 3-day period, demonstrating the positive impact of cold storage to stabilize virus infectivity.**
- **A separate time course study of LVV infectivity confirmed a much shorter half-life at 37°C (~6 hours) compared to 4°C (~300 hours)**

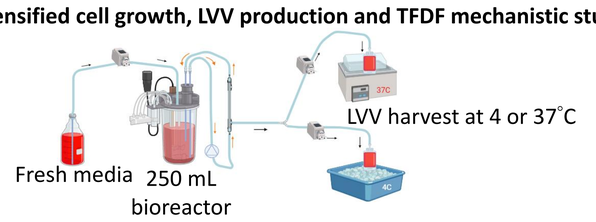
The second part of the study, still using a 250 mL bioreactor, showed a proof of concept of an integrated capture purification using a prototype affinity column directly connected to the TFDF permeate without further clarification.

- **A cumulative ~70% recovery yield of infective LVV particle was achieved compared to ~25% with a traditional batch process followed by harvest with depth filtration (~50% yield) and a capture step using anion exchange chromatography (AEX, ~50% yield)**

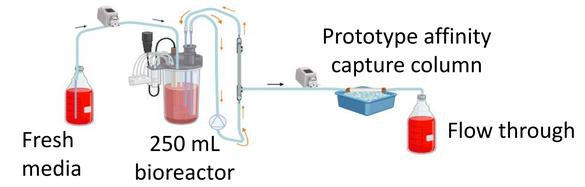
The third part of the study showed even further increased (1.5X) cell specific LVV productivity achieved from a 10 L TFDF-intensified process compared to the previous 2 L intensified process, leading to more than 5E11 total TU produced. This would be enough to produce >100 doses of a CAR-T therapy for a phase II or III clinical trial, while a >200 L batch cell culture would be required to produce the same number of doses.

Schematic workflow of this presentation

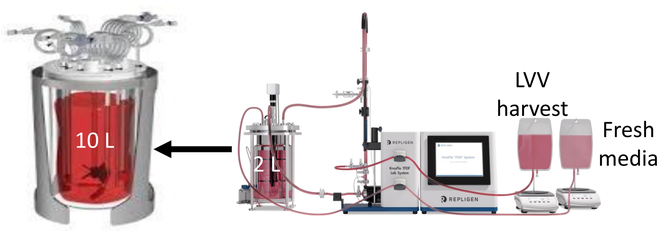
1. Intensified cell growth, LVV production and TFDF mechanistic study



2. Integrated affinity LVV capture



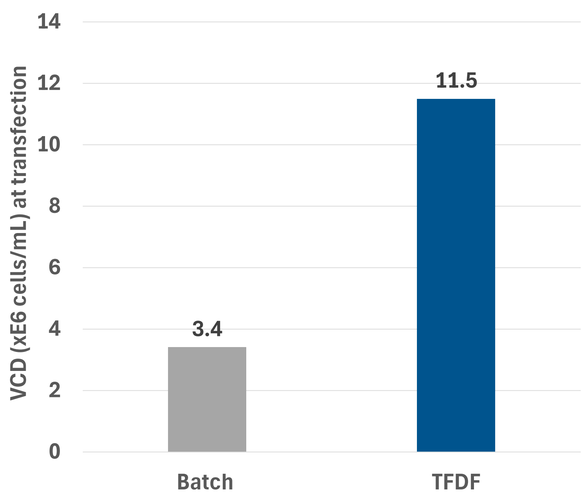
3. TFDF based scale up LVV production

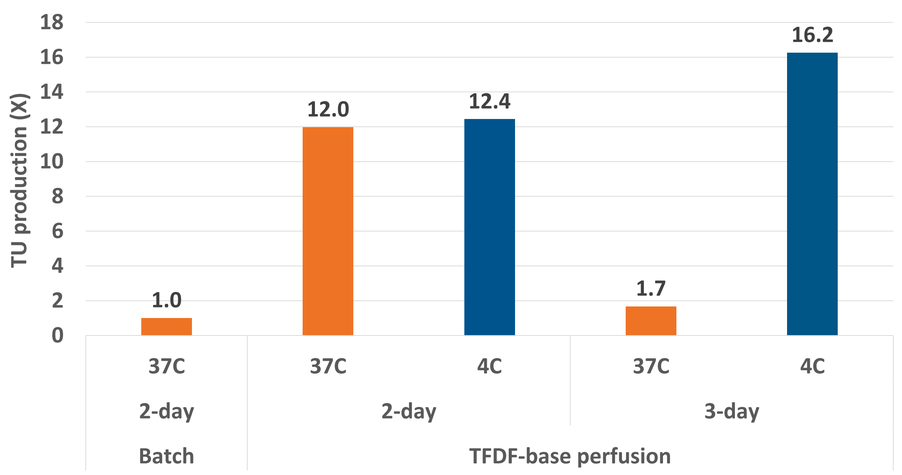


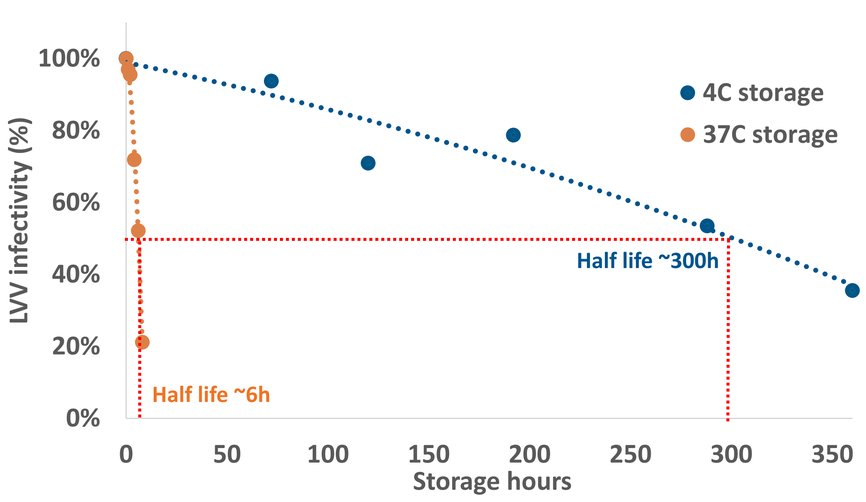
Cell culture conditions for integrated continuous LVV production

- **Scale down culture: TFDF (2-5 µm) prototype module (7 cm^2) connected via pump to 250 mL bioreactor, compared with batch process in same 250ml bioreactor.**
- **Scale-up culture: TFDF-30 flowpath (30 cm^2) integrated with a KrosFlo® TFDF Lab system connected to 2 L or 10 L bioreactors, compared with batch process in same bioreactors.**
- **Perfusion initiation 48 hours after inoculation at ~1.2 vessel volume per day (vvd)**
- **Cell inoculation at 1.1E6 cells/mL & transfect at ~4E6 or ~9-12E6 cells/mL for batch or TFDF-based perfusion processes**
- **Perfusion post transfection at ~1.2 vvd for continuous harvest**
- **TFDF permeate has low turbidity (<20 NTU), no need to clarification harvest**
- **Prototype affinity column directly connected to permeate for integrated continuous harvest**

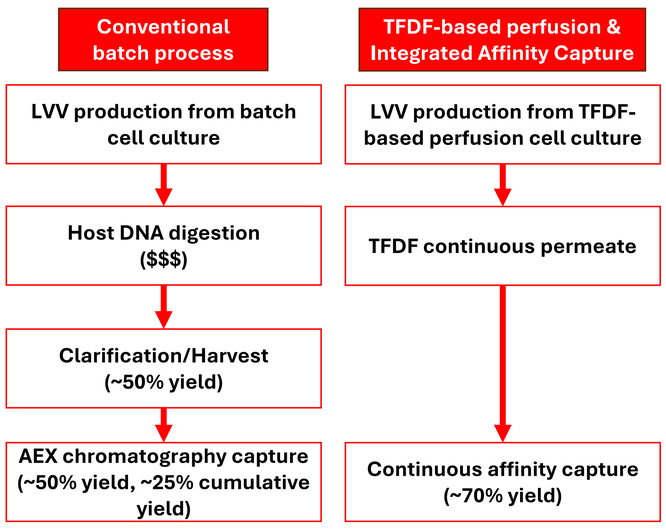
Characterization of TFDF-intensified LVV production at 250mL scale

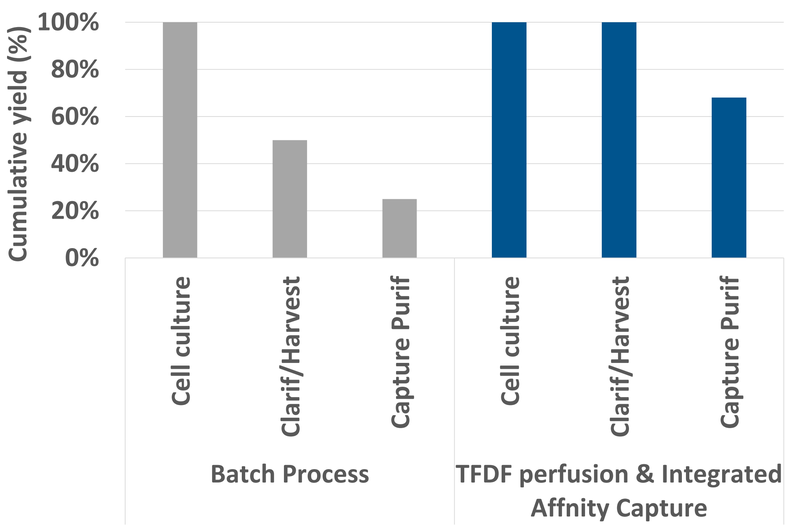




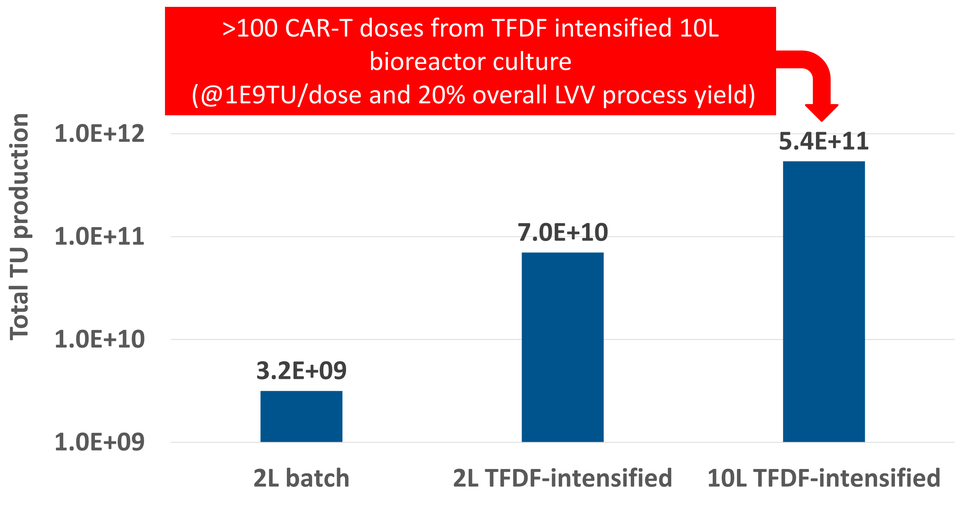


Integrated LVV production and affinity capture





TFDF-intensified LVV production scale-up



Conclusions

KrosFlo TFDF-based perfusion culture intensification enables

- **4X Increased number of cells and >4X increased cell specific LVV productivity**
- **Continuous cold temperature harvest, critical to stabilize LVV infectivity**
- **Integrated continuous affinity capture purification to further simplify the process and improve yield**
- **Production of >100 doses for CAR-T applications from a 10 L cell culture (vs >200 L for a batch process)**

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