Optimizing TFF Perfusion: Reducing or Removing Starling Flow to Improve Product Sieving in Mammalian Perfusion Processes

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Introduction

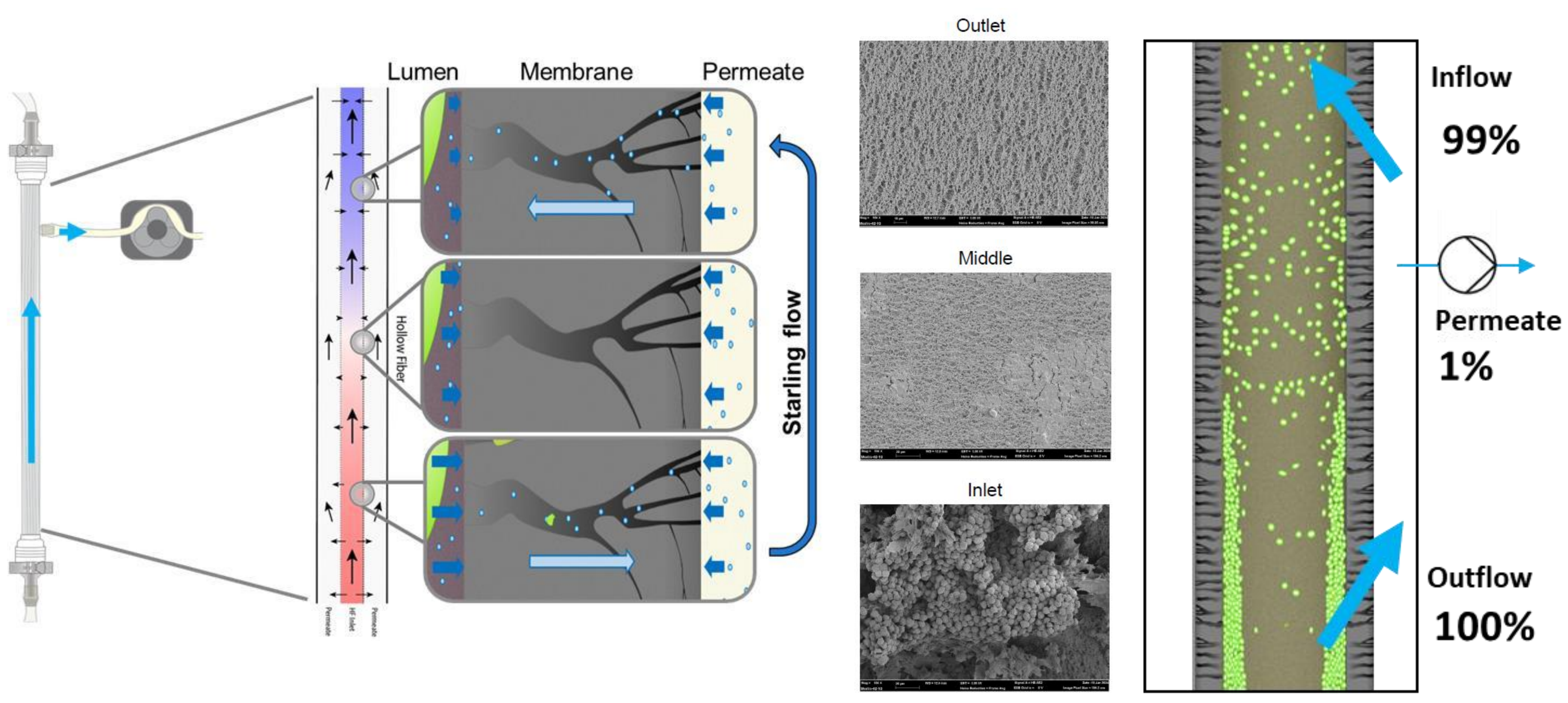
Hollow fiber-based cell retention has emerged as the preferred technology within the continuous manufacturing of complex therapeutic modalities. Nevertheless, filter fouling remains a major bottleneck in current manufacturing scenarios and scale-up. There lies a huge potential to improve performance by optimizing operating parameters and therefore better control the filtration process. This study investigates how to improve Tangential Flow Filtration (TFF) perfusion systems, which are often limited by membrane fouling and product retention—primarily due to the Starling flow effect caused by pressure drops in hollow fiber filters.

Theory

Introduction to Starling flow

Starling flow is caused by the axial pressure drop within the lumen of the hollow fiber module and results in a strong outflux of filtrate from lumen to permeate on the first half of the filter module, and a strong backflow (backflush) of filtrate on the second half of the filter module.

- To achieve a backflush, additional filtrate must be generated on the inlet side of the module, resulting in a recirculation (Starling flow)
- Starling flow can be up to 100x larger than the actual perfusion rate and represents a major factor influencing filtration performance

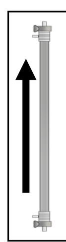


SEM picture reference: Dominik Schieman, "Product sieving understanding in different TFF operation modes in dynamic perfusion cultures" in "Recovery & Purification", BPI Vienna, (2024).

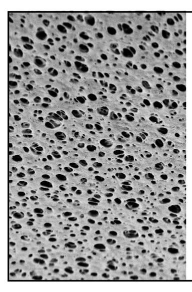
Strategies to reduce Starling flow

The amount of Starling flow is directly correlated to the pressure drop within the fiber lumen and the membrane resistance. Therefore, Starling flow can be influenced by many factors such as filter module choice (lumen ID, module length, pore size), crossflow and culture viscosity.

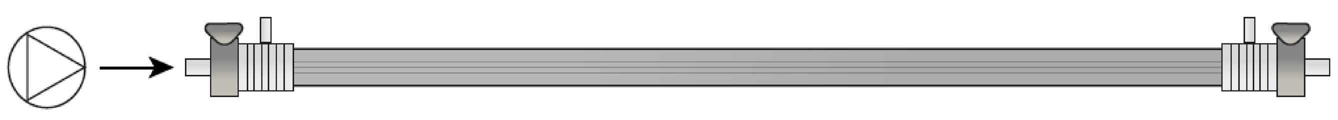
Filter Length:
- Increases pressure drop
- Limitation: restriction to shorter filters / parallel setups
- → **Reduce** filter length

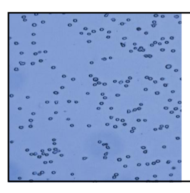


Crossflow Velocity:
- Increases pressure drop
- Limitation: restriction to low crossflows
- → **Reduce** Crossflow

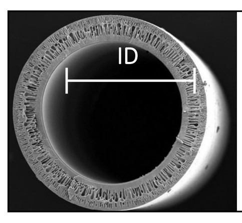


Membrane Resistance:
- Larger pores reduce membrane resistance
- Increase in Starling flow
- → **Reduce** pore size

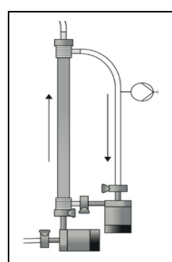




Culture Viscosity:
- Increases pressure drop
- → **Ideally** low viscosity



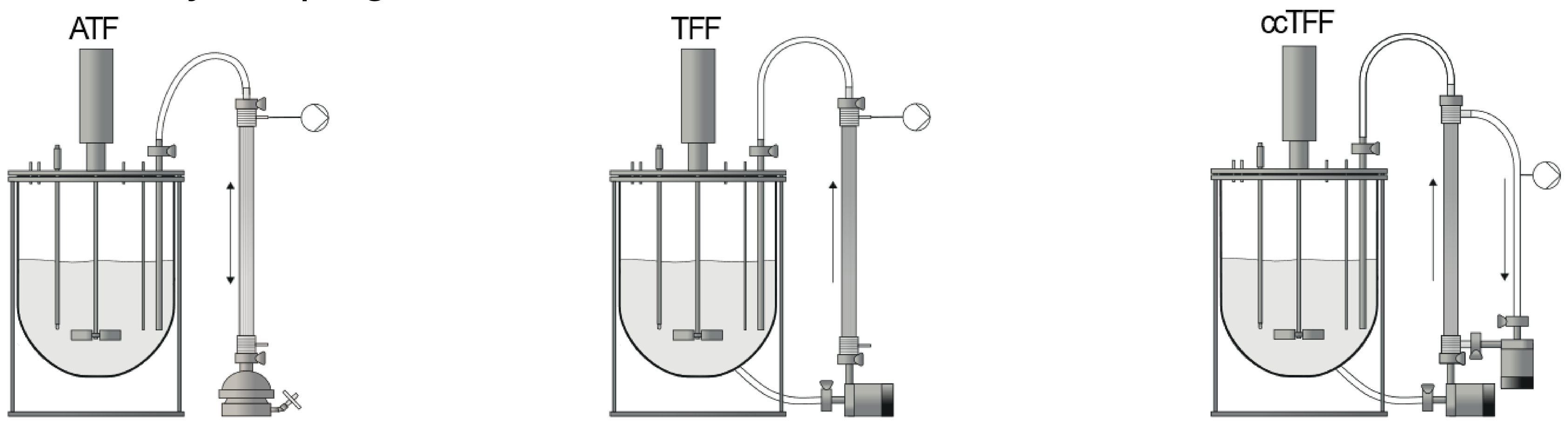
Fiber Lumen Diameter:
- Small diameters increase pressure drop
- Large diameters reduce membrane surface
- → **Increase** lumen diameter



Co-current Filtrate Flow:
- Apply co-current filtrate flow
- ccTFF or scTFF

Experimental Approach

Alternating Tangential Flow (ATF) was used as a reference system to compare against the performance of different TFF configurations, equipped with levitating centrifugal pumps. The comparison was done by running the different perfusion systems for a period up to 30 days. To reduce the Starling flow, the TFF system was operated at a low wall-shear rate regime. As an additional measure the co-current TFF setup was introduced and tested by keeping the same conditions.



ATF:
- 1 membrane pump
- Alternating flow

TFF:
- 1 centrifugal pump
- Unidirectional flow

Co-current TFF
- 2 centrifugal pumps
- Co-current filtrate flow

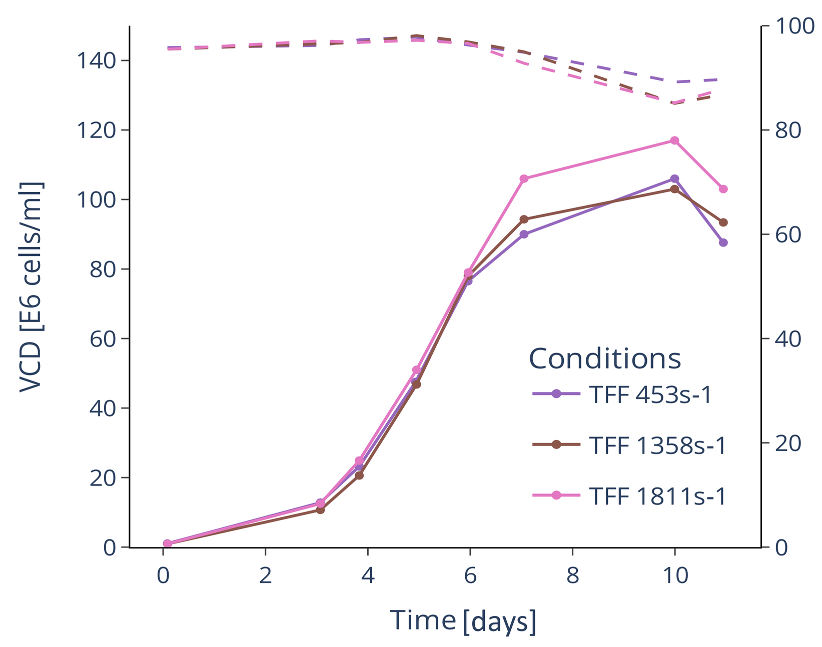
Results and Discussion

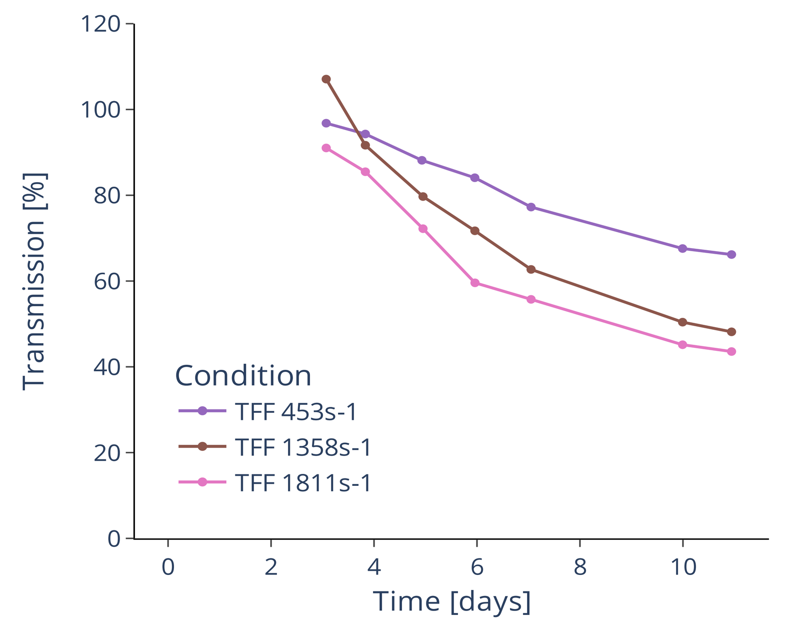
Cell Growth and Viability: All TFF systems supported rapid cell growth up to ~100E6 cells/mL within 10 days, with TFF 1358s^{-1} and TFF 1811s^{-1} slightly outperforming TFF 453s^{-1}. Viability remained over (>80%) across all conditions.

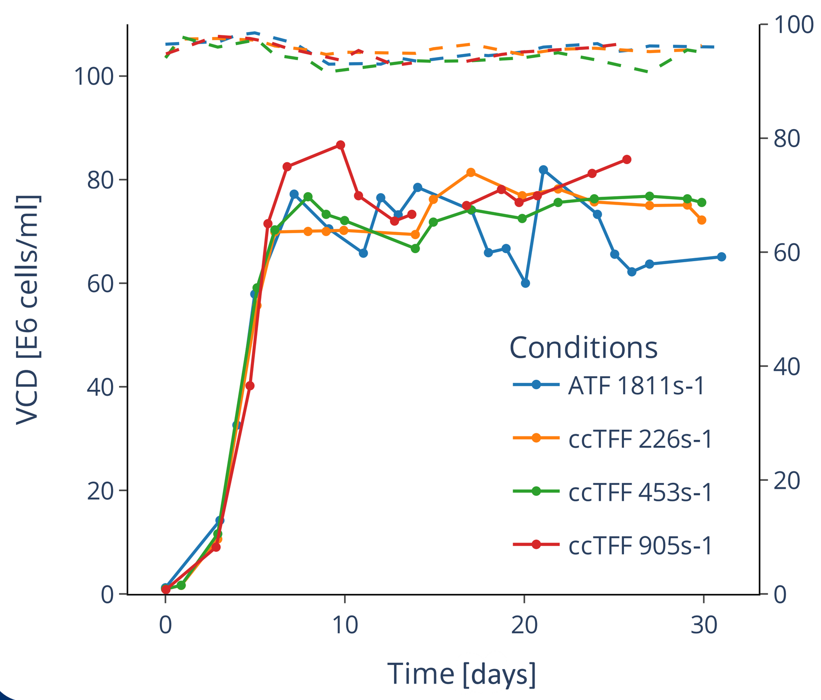
Filter Transmission in TFF: Transmission declined over 10 days in all TFF setups. TFF 453s^{-1} maintained the best performance (~70%), while TFF 1811s^{-1} dropped to ~45%, suggesting increased fouling or retention.

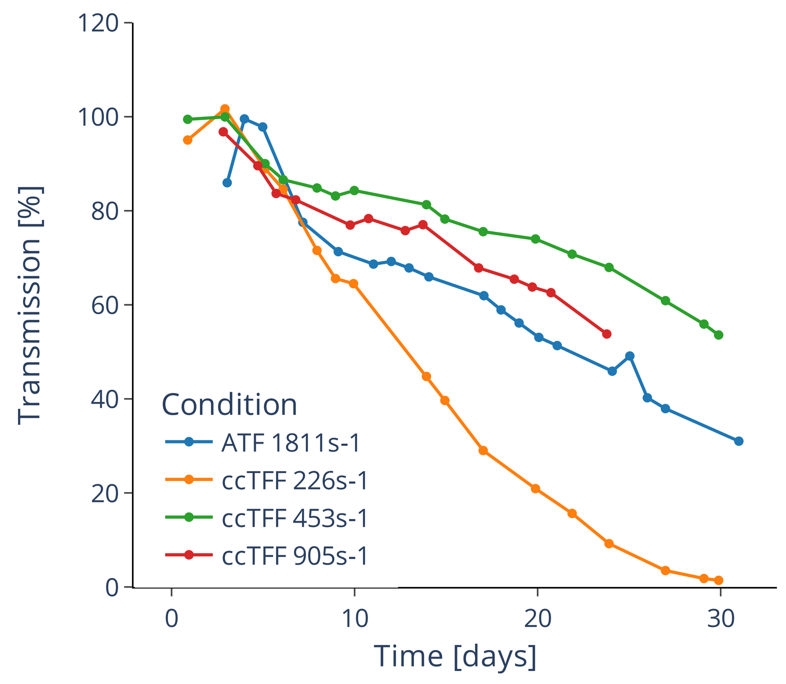
ATF vs. ccTFF Systems: In extended runs, ATF and ccTFF systems rapidly reached target VCDs and maintained high viability. ccTFF 905s^{-1} showed the most stable growth and consistent performance.

Long-Term Filter Stability: Up to 30 days, ccTFF 905s^{-1} retained the highest filter transmission (~60%), outperforming ATF 1811s^{-1} and other configurations, indicating better long-term robustness.









Conclusion and Outlook

Whereas the standard TFF system performance could be significantly improved by reducing crossflow, and thereby minimizing Starling flow, co-current filtrate flow systems improved performance even more. With up to 60% transmission maintained up to 30 days and an 8% increase in product yield, ccTFF shows strong potential to enhance volumetric productivity and reduce operational burdens such as cleaning and turnover times.