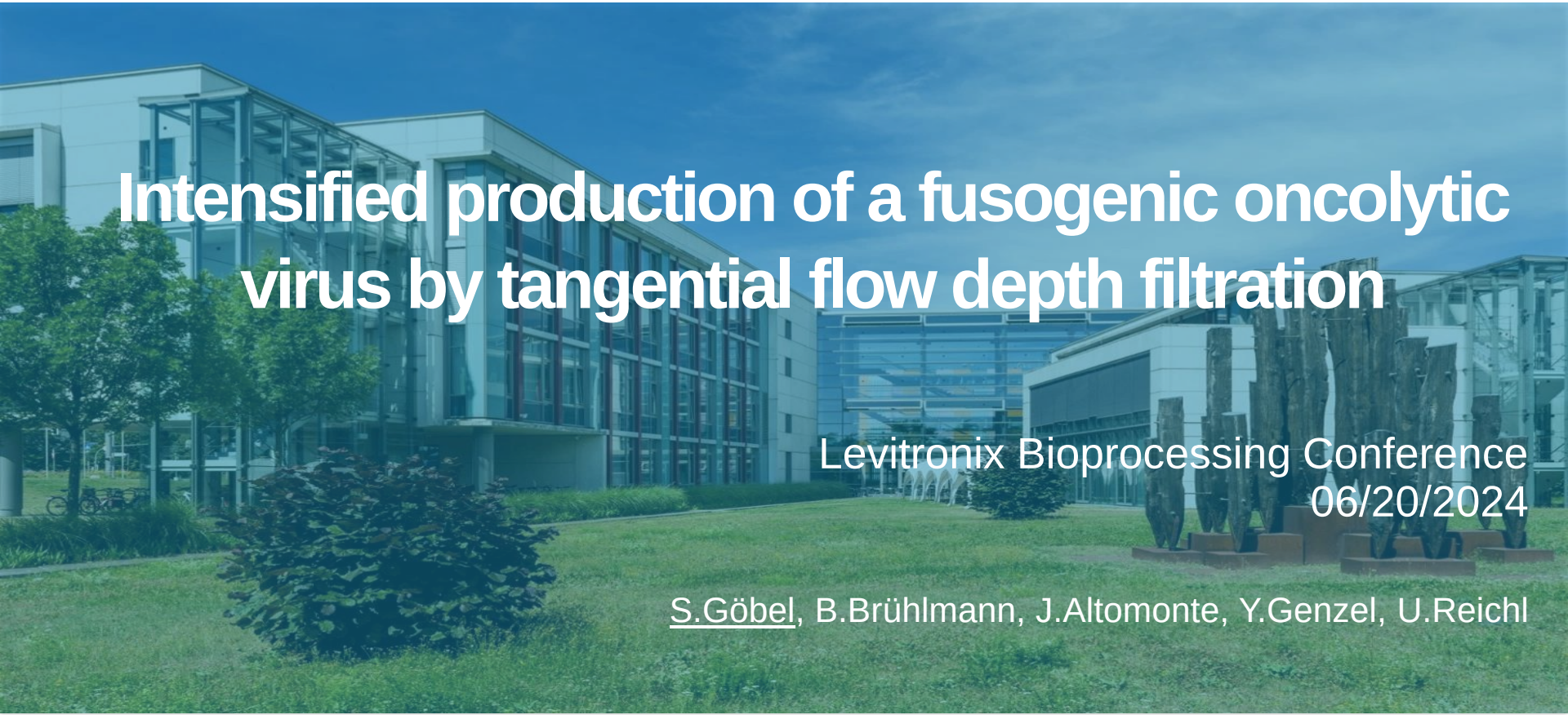

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BPE BIOPROCESS ENGINEERING

Intensified production of a fusogenic oncolytic virus by tangential flow depth filtration

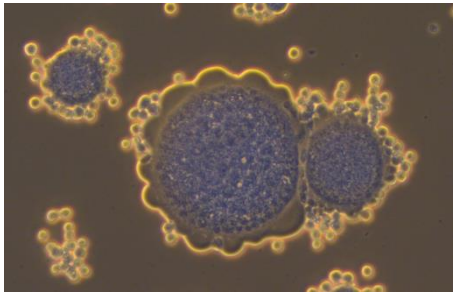
Levitronix Bioprocessing Conference
06/20/2024

S.Göbel, B.Brühlmann, J.Altomonte, Y.Genzel, U.Reichl

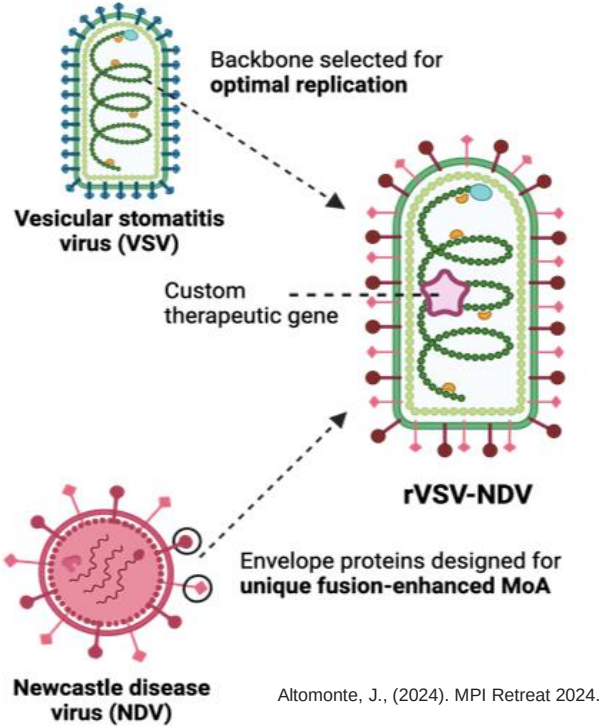
Fusogenic oncolytic rVSV-NDV

- Fusogenic rVSV-NDV is a promising example for an oncolytic rVSV platform
- Increasing demand and high input doses required for rVSV-based therapies
- Formation of large multi-nucleated syncytia after infection (up to 120 µm)

→ Proof-of-concept production with hollow-fiber or TFDF-based retention in a perfusion mode



Göbel et al., (2023). *Appl Microbiol Biotechnol.*



Altomonte, J., (2024). MPI Retreat 2024.

Hollow-fiber vs TFDF membrane

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*Values for 0.22 µm PES shown as mean±STD of n=5

0.5-1 mm

up to 0.2 mm

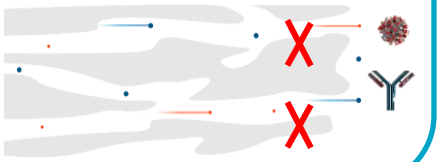
anisotropic

HFM

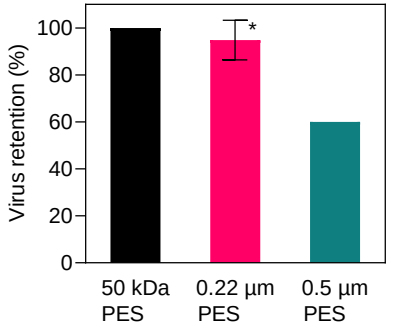
Modified polyethersulfone fibres

Pore size

0.2 – 0.65 µm: retention of large proteins and viruses

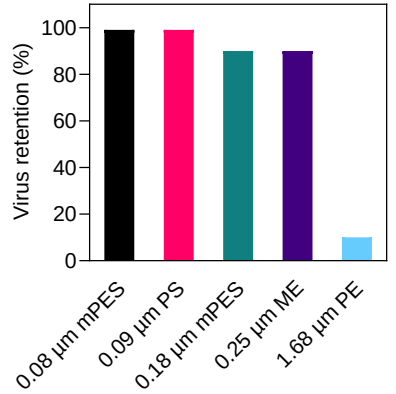


Influenza A virus



ATF

Yellow fever virus



TFF

Drawbacks of virus retention

- High residence time of virus particles → reduced stability
- Degradation of infectious virus particles
- No option for process integration

Genzel et al., (2014). *Vaccine*
Wu et. al., (2021), *Appl Microbiol Biotechnol.*
Pelz et al., (2024), *Appl Microbiol Biotechnol.*
Nikolay et al., (2020), *Biotech. & Bioeng.*

Hollow-fiber vs TFDF membrane

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1 mm

up to 0.2 mm

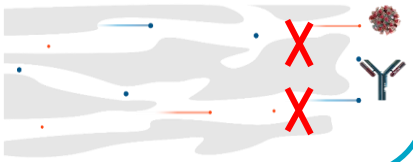
anisotropic

HFM

Modified polyethersulfone fibres

Pore size

0.2 – 0.65 µm: retention of large proteins and viruses

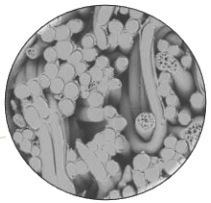


TFDF

4.6 mm

5 mm

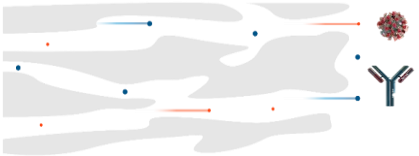
isotropic

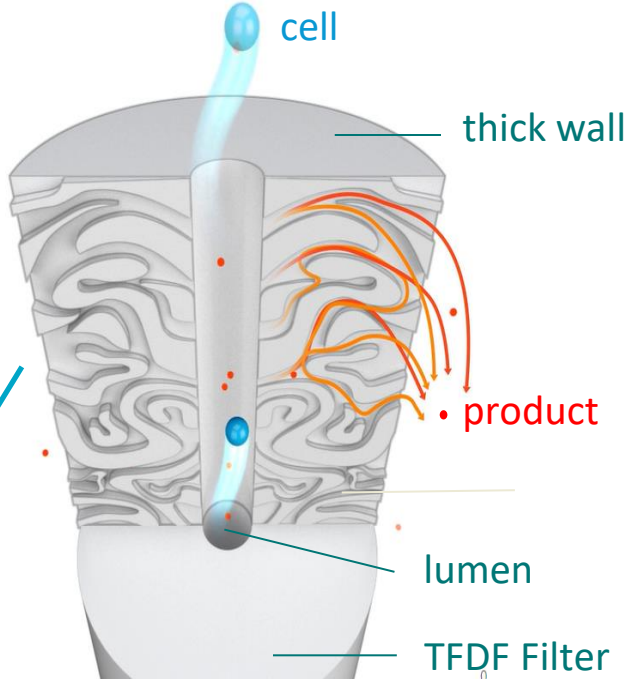


Polypropylene/Polyethylene terephthalate fibre

Pore size

2 - 5 µm: easily transmits large proteins and viruses





KrosFlo TFDF System unique filtration technology

Combines the benefits of tangential flow and depth filtration

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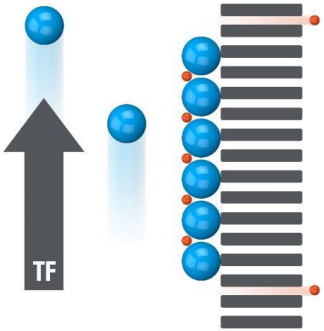
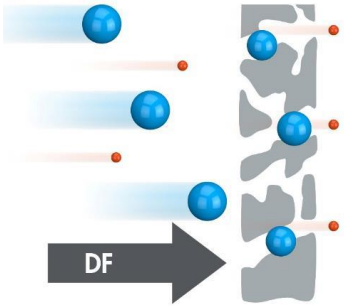
Cells and cell debris
Protein or virus



Tangential Flow

High cell density
Low product collection

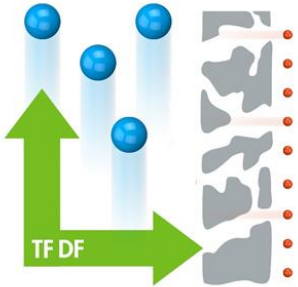
+



Depth Filtration

Low cell density
High product collection

=



Tangential Flow Depth Filtration

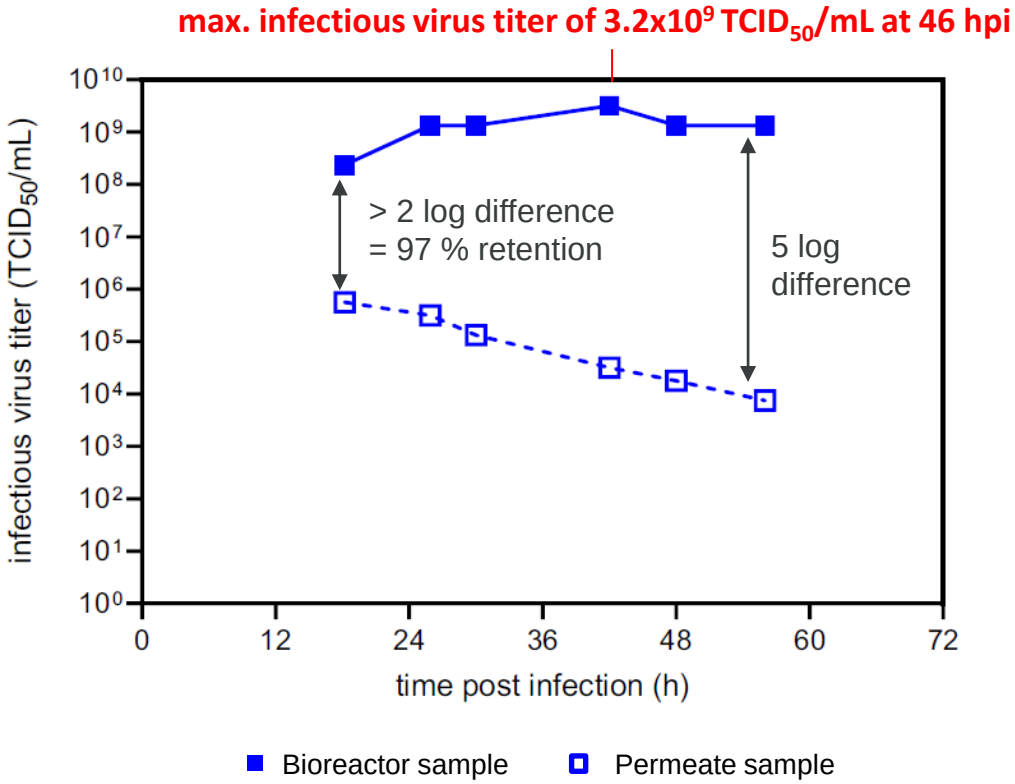
High cell density
High product collection

TFDF processes high cell densities with high product transmission

Figure kindley provided by Repligen

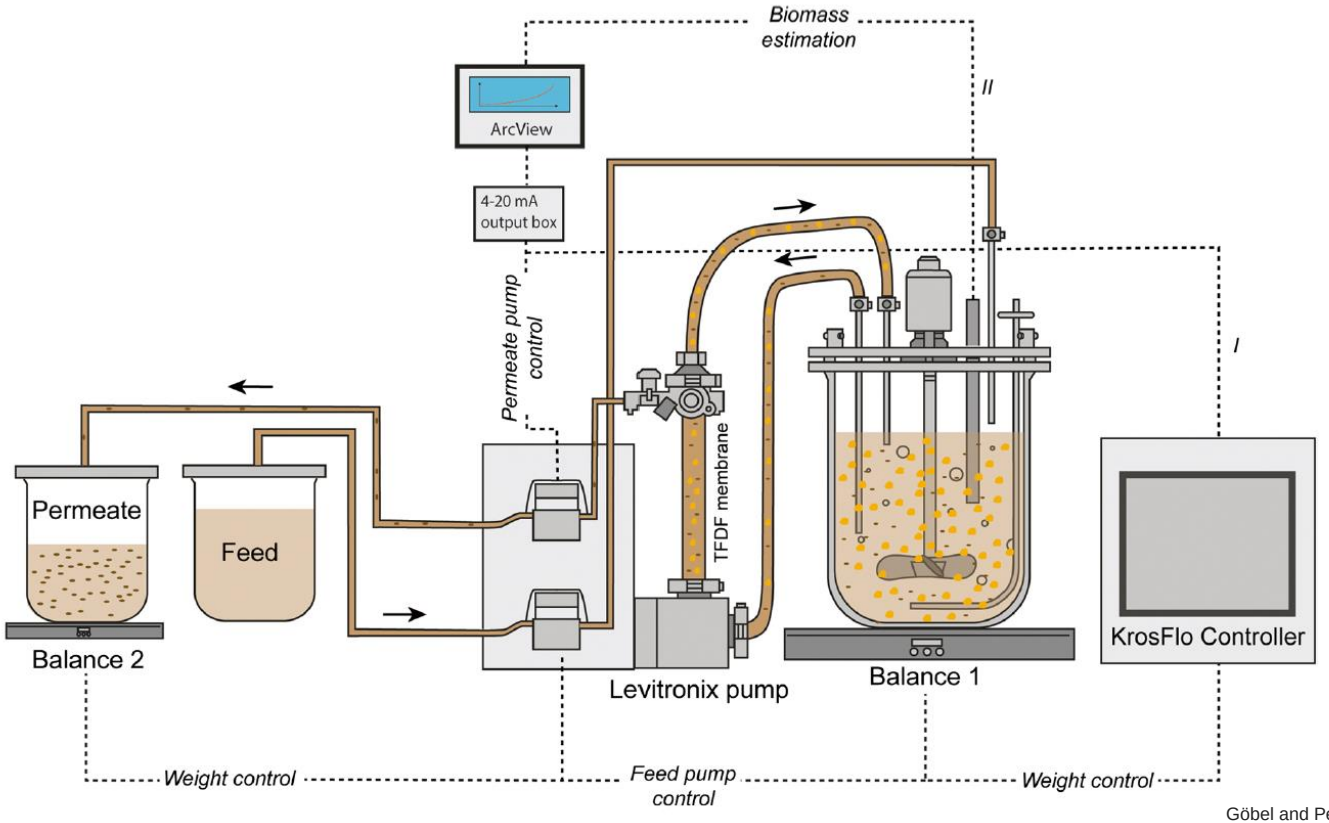
High retention of infectious rVSV-NDV virus for HFM

BHK-21 cells in perfusion ATF mode; 0.65 µm HFM



TFDF setup for perfusion cultivations of rVSV-NDV

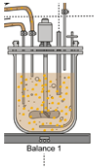
Manual (I) or capacitance-based control (II) of perfusion rate

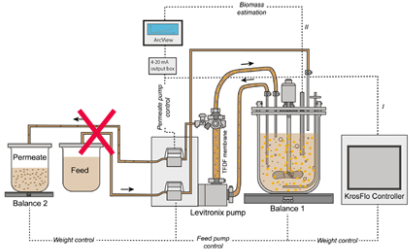


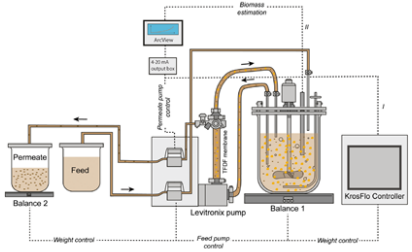
Göbel and Pelz. et al., (2024) *Appl Microbiol Biotechnol*

BHK-21 rVSV-NDV in STR and TFDF

Workflow of perfusion, infection, harvest and clarification







batch growth	perfusion start	perfusion stop	media exchange	infection	perfusion restart	cont. harvest and clarification
Inoculation with 0.8x10^6 cells/mL and batch growth until 4.0x10^6 cells/mL	Start perfusion with 130 / 168 pL/cell/day once 4.0x10^6 cells/mL are reached	Stop perfusion when 14.0x10^6 cells/mL were reached	Complete media exchange (reactor volume) with 10 mL/min flow	Infected with MOI of 1E-4. Temp. reduction to 34°C and paused for 1-2 h	Maintained perfusion at 1.8 VVD	clarified permeate collected cont. in PET bottles, final harvest with CDC
- 120	- (46-51)			0	1-2	48-60

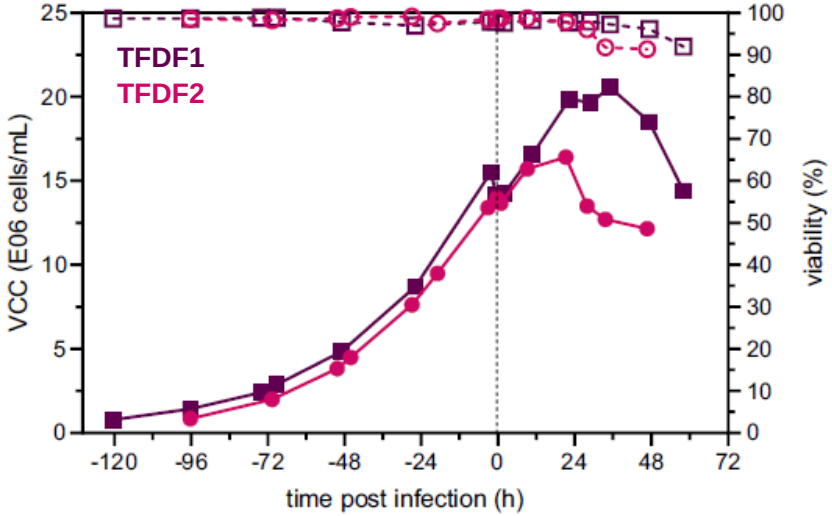
time post infection (h)

>5X increase in VCC at infection with perfusion over batch

Cell growth of BHK in STR in perfusion mode using TFDF for cell retention

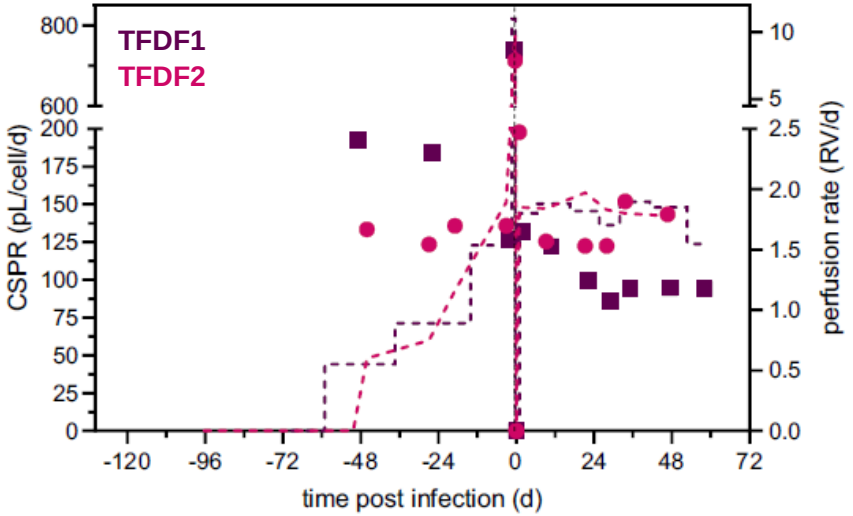
TFDF1 = manually controlled perfusion rate

TFDF2 = feed-back loop perfusion rate (capacitance probe)



VCC (10^6 cells/mL) TFDF1 — viability (%) TFDF1

VCC (10^6 cells/mL) TFDF2 — viability (%) TFDF2



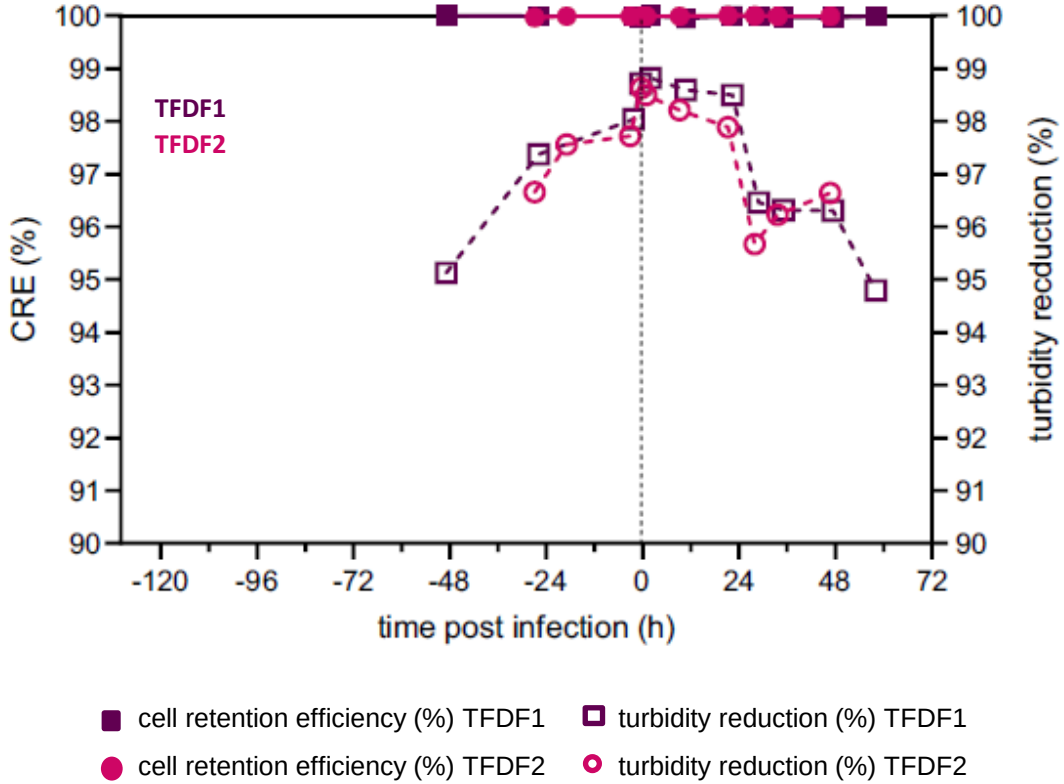
CSPR (pL/cell/d) TFDF1 — Perfusion rate (RV/d) TFDF1

CSPR (pL/cell/d) TFDF2 — Perfusion rate (RV/d) TFDF2

15% reduction of medium consumption

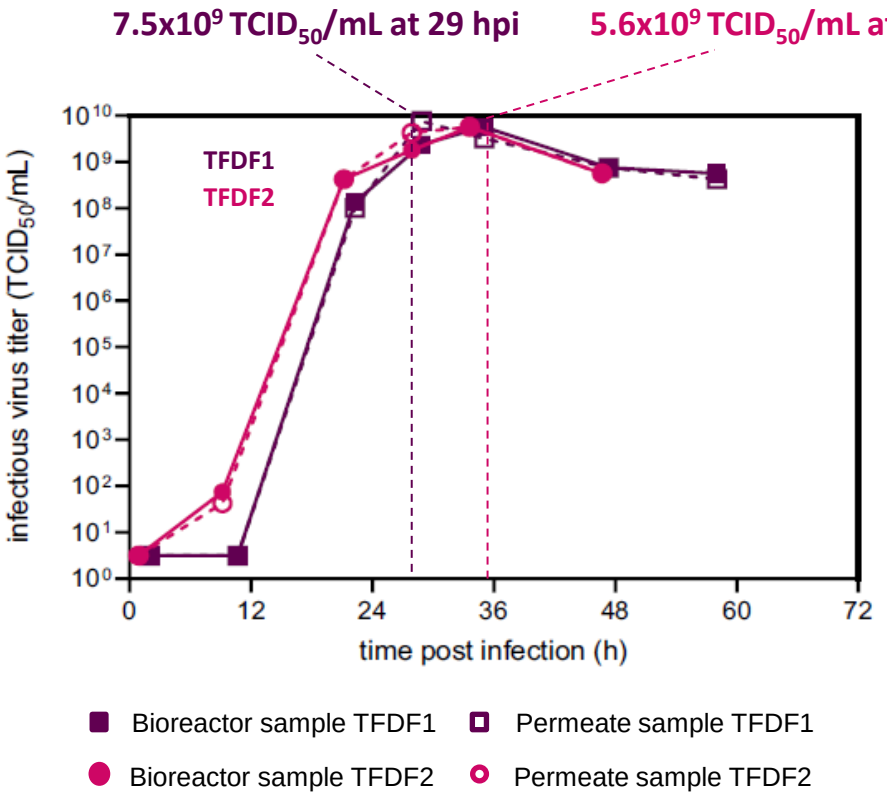
High turbidity reduction, 100% cell retention with TFDF

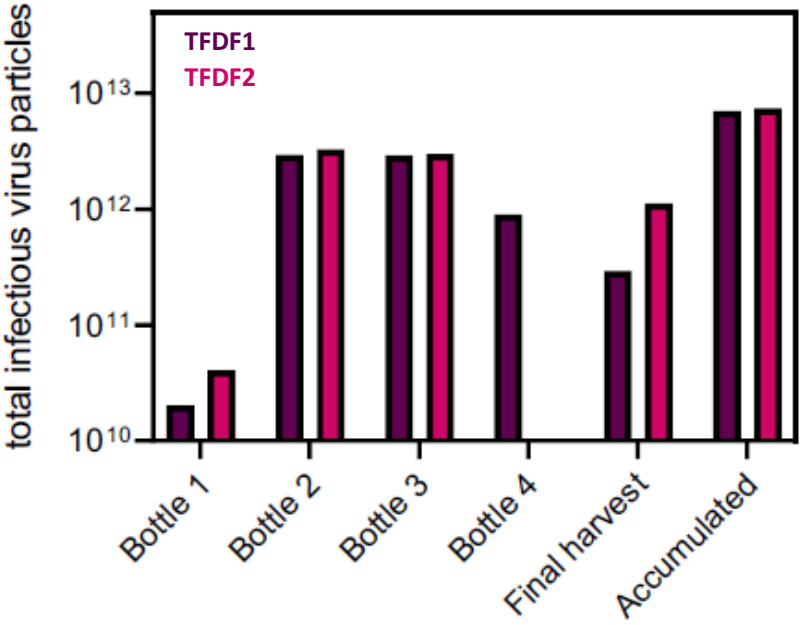
Harvest/clarification of BHK-21 rVSV-NDV in STR



Low rVSV-NDV retention and reproducible productivity

Infectious virus titer ($TCID_{50}$/mL) rVSV-NDV in BHK-21 in STR





Summary

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- >**97% virus retention** when using 0.65 µm mPES HF membranes
- POC of TFDF for perfusion and continuous rVSV harvest with clarification system worked very well
- Intensified TFDF processes achieved **highest reported infectious virus titers** of 5.6 - 7.5x10^9 $TCID_{50}$/mL so far
- Compared to optimized batch: 5-6x increased VCC but **> 11x increased infectious virus titer, 2x improved CSVY and STY by 460 % (5.6-fold)**
- Compared to other perfusion systems: > **1.5 - 3x increased infectious virus titer**, always more than **2x increased VVP and STY**
- No impact of syncytia formation on performance of TFDF system

Intensify multiple upstream and downstream unit operations

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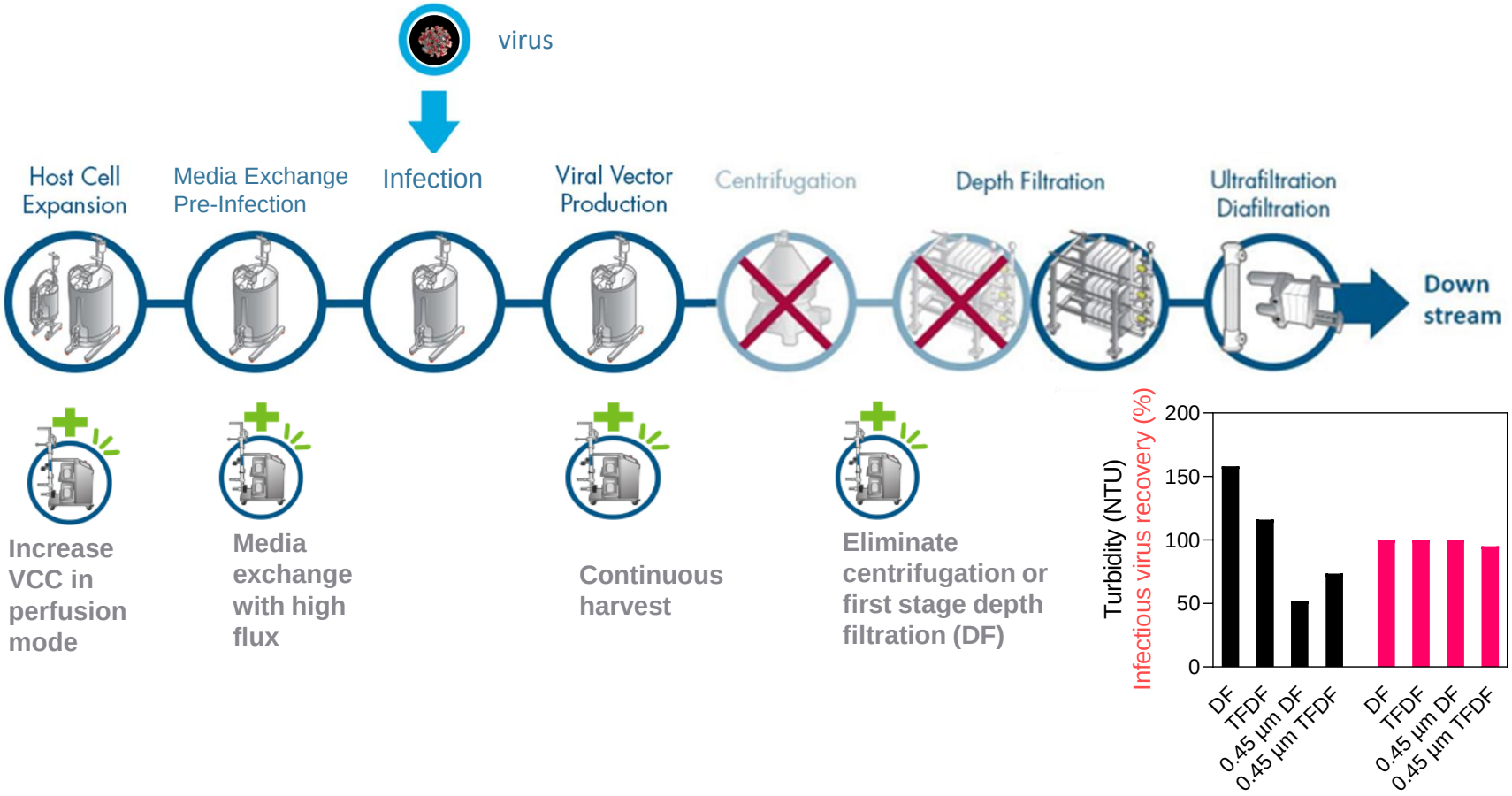

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Rachel Legman

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Thank you for your attention
Any Questions?