MAX PLANCK INSTITUTE FOR DYNAMICS OF COMPLEX TECHNICAL SYSTEMS MAGDEBURG

BPE BIOPROCESS ENGINEERING

OTTO VON GUERICKE UNIVERSITÄT MAGDEBURG

Production of defective interfering particles in a high cell density perfusion cultivations with continuous virus harvesting

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INTRODUCTION

Since many years, defective interfering particles (DIPs) derived from influenza A virus (IAV) have been discussed for their use as antivirals [1]. DIPs are virus mutants harboring an internal deletion in one of their viral RNA (vRNA) segments. Due to these deletions, DIPs can only replicate in the presence of infectious standard virus (STV), compensating for the missing gene function. In a co-infection, the defective vRNA interferes with STV genome replication and stimulates the immune response, and thus has therapeutic potential. In the present study, we established an automated perfusion process for production of a DIP, called DI244, using a VHU® Perfusion system comprising tubular membrane modules for cell retention and for continuous virus harvesting into the permeate.

GENETIC PROPERTIES OF DI244 AND MDCK-PB2

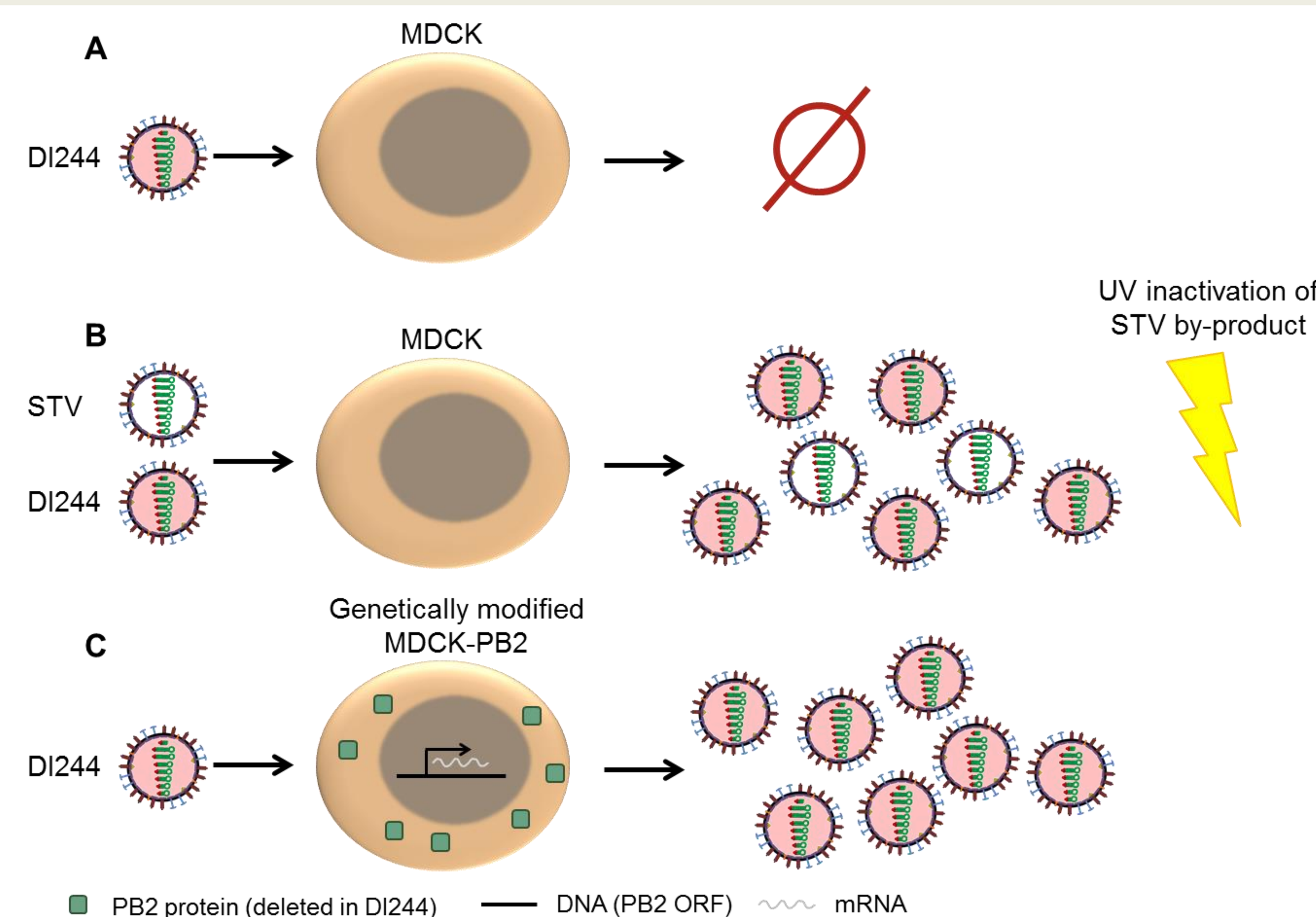
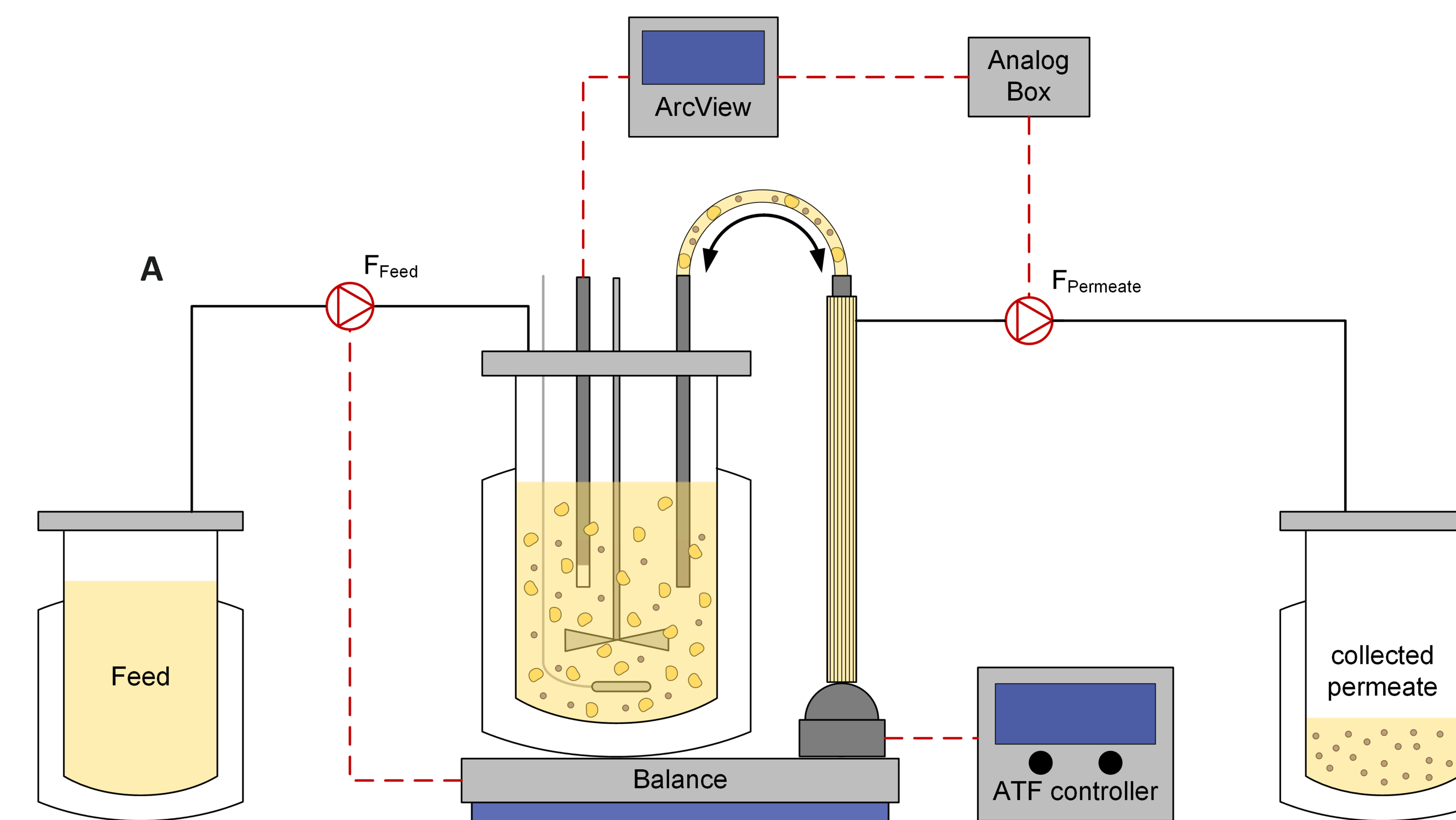


Fig. 1 Cell culture-based production of pure DI244 particles. (A) Infection of MDCK cells with pure DI244 will not result in replication due to the deletion in segment 1 vRNA of DI244 (encoding for PB2). (B) Co-infections of MDCK cells with STV and DI244 will result in DI244 replication. Here, infectious STV particles will be produced as well, which need to be inactivated by UV irradiation. (C) Genetic modification of MDCK cells to express the "missing" PB2 results in DI244 replication. Here, genetically modified suspension (MDCK-PB2(sus)) were used to produce pure DI244 particles, whereas adherent MDCK cells (MDCK-PB2(adh)) were used for the plaque assay.

- DI244 particles lack the genetic information for the viral protein PB2
- MDCK-PB2 cells provide the missing PB2 protein and allow DI244 replication [2]
- No STV contaminating the product is formed and no UV irradiation is necessary

DI244 PRODUCTION IN A PERFUSION PROCESS



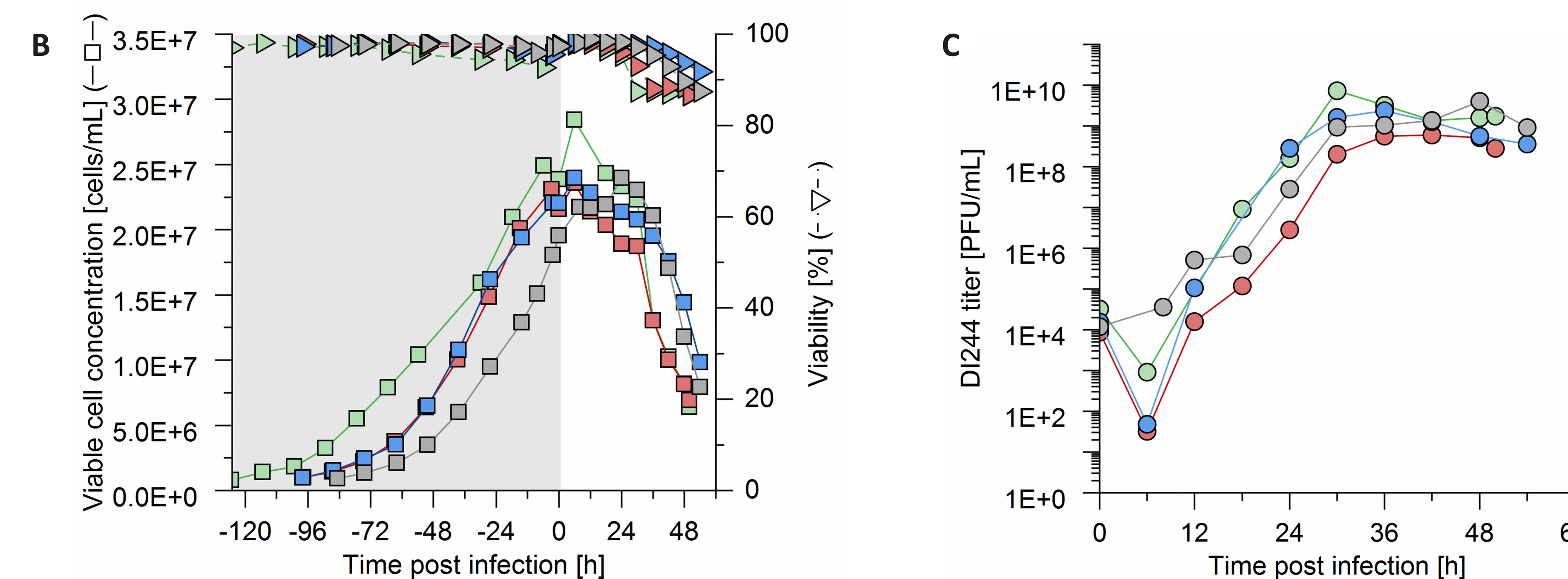


Fig. 2 DI244 production in perfusion cultivations. (A) Bioreactor setup for perfusion cultivations (1 L-stirred tank glass bioreactor with a working volume of 700 mL coupled to an ATF2 system). On-line monitoring of viable cell volume via a capacitance probe (Hamilton, Arc View). Depending on the membrane used for cell retention, virus particles could be either found exclusively in the STR or additionally in the collected permeate (see fig 4). (B and C) MDCK-PB2 cells cultivated in four perfusion cultivations were infected at a VCC of 20.0E+6 cells/mL with a pure DI244 seed virus. (B) VCC and viability, (C) DI244 vRNA level.

- An automated perfusion process for DI244 production was established using the capacitance signal
- VCC up to 28.4E6 cells/mL and DI244 titer up to 7.4E9 PFU/mL were reached [3]

CONTINUOUS VIRUS HARVESTING

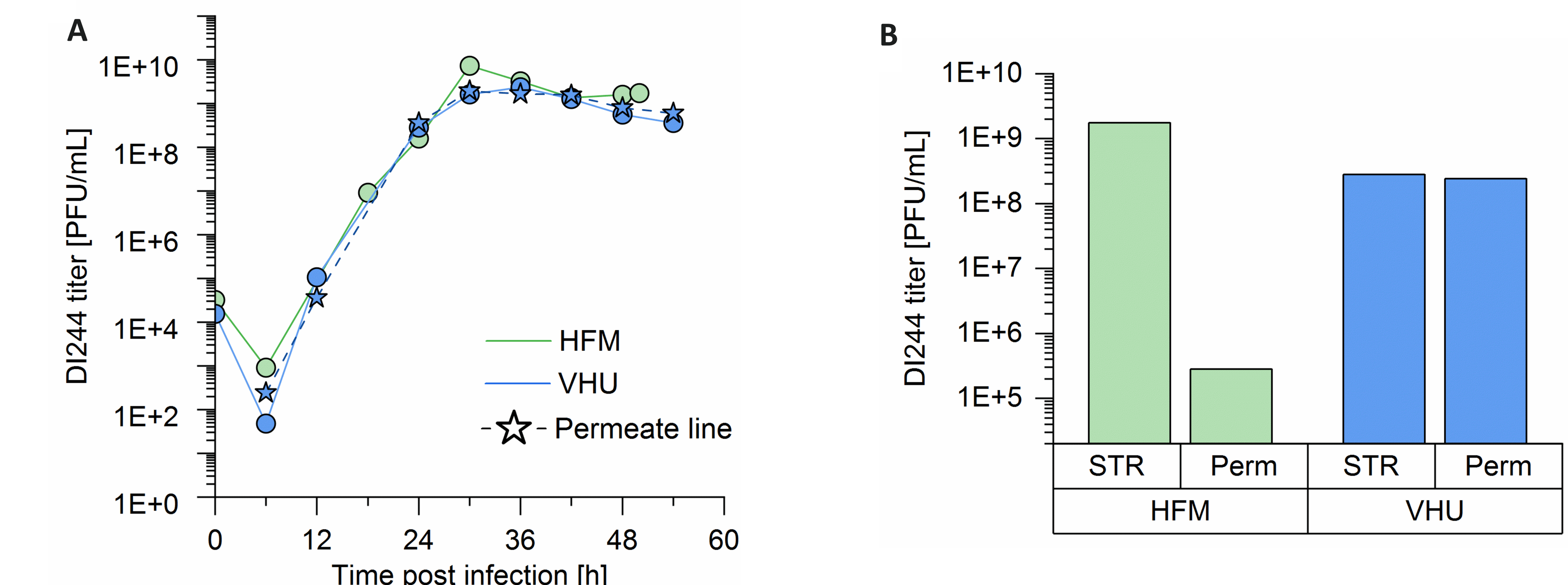


Fig. 3 Comparison of different cell retention membranes. MDCK-PB2 cells were cultivated in perfusion cultivations using either a commonly used hollow fiber membrane (HFM; pore size 0.2 µm) or the virus harvest unit from Artemis Biosystems (VHU®; pore size ~10 µm) for cell retention. (A) DI244 titer. Solid lines correspond to virus titer in the STR, dashed lines with star symbols to the virus titer in the permeate line. (B) DI244 titer in STR and permeate line (Perm) at time of harvest (48/52 hours post infection).

- A tubular cell retention membrane (VHU®) allowed continuous virus harvesting during the perfusion cultivation [3]

CONCLUSION AND OUTLOOK

- MDCK-PB2 cells allowed propagation of pure DI244 in absence of infectious STV
- A automated perfusion process was established yielding VCC up to 28.4E6 cells/mL and DI244 titer up to 7.4E9 PFU/mL
- The VHU membrane, in contrast to commonly used hollow fiber membranes, allowed continuous virus harvesting during the cultivation

REFERENCES

[1] Dimmock et al. (2012) Antiviral Res 96(3):376-85
[2] Bdeir et al. (2019) PLOS ONE.0212757
[3] Hein et al. (2021) *Appl Microbiol Biotechnol* 105, 7251–7264