INTENSIFIED ENVELOPED VECTOR PRODUCTION USING CONTINUOUS PERFUSION BIOPROCESSING

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ABSTRACT

A great demand of vectors for gene therapy and vaccination drives development of breakthrough technologies for vector production beyond established technologies for non-enveloped gene transfer vectors such as AAV. New modalities in the cell and gene therapy industry also require innovation in process technologies and manufacturing methods to keep pace. GenVivo addresses this need by evaluating tangential flow depth filtration (TFDF) technology for the perfusion process to produce enveloped vectors from a human-derived cell line in single use 3L bioreactors. Although alternative tangential flow (ATF) and tangential flow filtration (TFF) have been applied to the perfusion process for producing monoclonal antibodies due to their large membrane surface, ease to implementation, high recovery of viable cells, and a small footprint, fouling of the surface of ATF and TFF fibers associated with the build-up of cells, cell debris, particulates and the extracellular secretive products are one of the challenging issues for a long perfusion process. TFDF uses a depth filter with a micro-meter pore size in a tangential flow mode, realizing a high efficiency in cell retention and circumventing the fouling issue.

INTRODUCTION

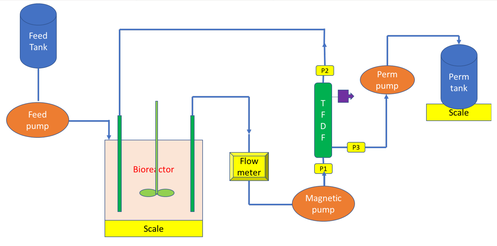
- Perfusion processes are being developed for monoclonal antibodies:
  - Economical due to improved productivity; small capital cost; reduced footprints; and boosted flexibility for diverse products.
  - Better product quality due to controlled culture environment; pseudo steady state operation; shorter residence time; higher cell viabilities; lower levels of impurities; and continuous harvest of unstable products.
  - Biggest challenge with ATF and TFF devices is potential fouling.
- Cell and Gene Therapy (CGT) sectors are starting to embrace perfusion processes:
  - But, there are still some challenges due to the fragile nature of the enveloped vectors and their large size
  - A newer filter system known as the TFDF holds great potential for CGT products:
    - Containing a depth filter with a micro-meter pore size, 2-5mm
    - With similar properties as the ATF and TFF, providing the tangential flow mode capability
    - Potential for providing a higher efficiency in cell retention and better product quality
    - And potential for mitigating the fouling issue during the perfusion process

METHODOLOGY

The human-derived suspension HEK293 cells were inoculated into bench-scale bioreactors, which were controlled via BIOSTAT-DCU II (Sartorius Stedim Biotech) at a power per unit volume of 10-30 W/m^3, with setpoints for temperature of 37°C, pH of 7.0 and DO of 40-50%. TFDF filters (30 cm^2) at a pore size of 3-5 micron (Repligen, CA) were used for the perfusion process, and the shear rate across the filter surface was kept at 2000 s^{-1}. Five bioreactor runs were carried out to evaluate the TFDF filter for the perfusion process. Various permeate flow rates in the range of 0.5- 5.0 VVDs (volume per vessel volume per day) were applied.

Table 1. Experimental details for five perfusion processes with TFDF filters.

Bioreactor run #	Bioreactor	Working Volume (L)	Inoculation Viable Cell (vc) Density (vc/mL)
1	Mobius 3 L	2.4	2.1x10^6
2	Mobius 3 L	2.4	1.8x10^6
3	Distek Bioreactor	2.0	2.7x10^6
4	Mobius 3 L	2.0	9.2x10^6
5	Distek Bioreactor	2.0	5.0x10^6



Scheme 1. Experimental setup for TFDF perfusion process.

RESULTS

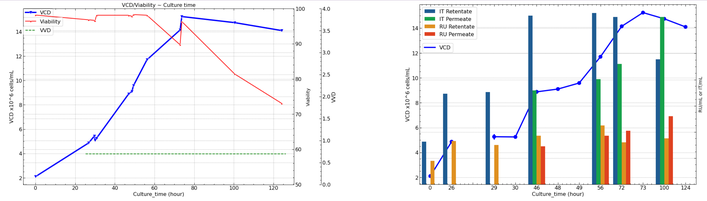


Figure 1. Constant VVDs for the first perfusion run. Viable Cell Density (VCD) and percent viability (left) and physical titer (RU) and infectious titer (IT) of permeate and retentate (right) at a constant VVD of 0.7. Equivalent RU and IT values are seen between the permeate and retentate, and no fouling is observed. The run was terminated due to a low viability, which could be due to insufficient nutrient supply or inadequate dissolved oxygen.

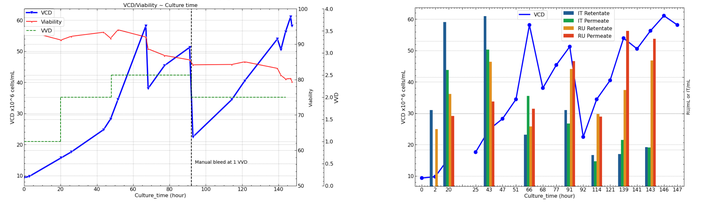


Figure 2. Various VVDs for the perfusion processes in a Mobius Bioreactor up to ~140 hours days (day 6) without fouling (Run #4). VCD and percent viability (left) and physical titer (RU) and infectious titer (IT) of permeate and retentate (right) at an increasing VVD as the densities increases. After manual bleed at 1 VVD, the VVD is reduced to 2.0. A cell density up to 50-60 x10^6 vc/mL is achieved before and post bleed. Equivalent RU and IT values are seen between the permeate and retentate, while infectious titers are reduced from 91 hours onwards. See discussion for possible reasons.

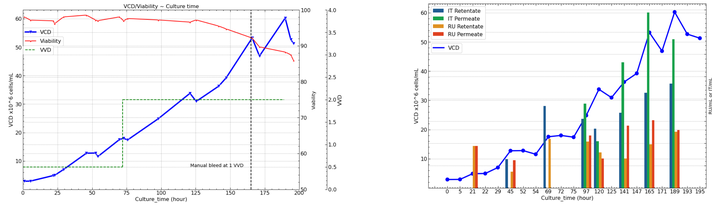


Figure 3. Various VVDs for the perfusion processes in a Distek Bioreactor up to 195 hours (day 8) without fouling (Run #3). VCD and percent viability (left) and physical titer (RU) and infectious titer (IT) of permeate and retentate (right) at the VVD of 0.5 and 2.0. A cell density of 50-60 x10^6 vc/mL is achieved before and post manual bleed of 1 VVD on day 7. Equivalent RU values are seen between the permeate and retentate, while infectious titers in the permeate are higher than that in the retentate, suggesting removing the vectors during the perfusion process could protect the vector infectivity.

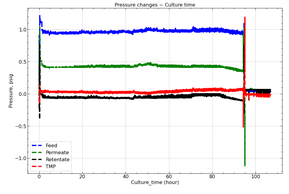


Figure 4. Pressure monitoring on the feed line, retentate line, permeate line and the calculated Trans Membrane Pressure (TMP) for Run #1. There are no pressure change in the feed pressure, retentate pressure, and permeate pressure for all five runs. The TMP is quite stable for all five runs, suggesting no filter fouling for up to 10 days, even at a cell density of 60 x 10^6 vc/mL

DISCUSSION

- **Perfusion Process:**
  - The cell density is exponentially increased after perfusion is implemented and a high viability is maintained in the bioreactor (Figure 1-3).
  - A constant VVD of 0.7 may be sufficient for a VCD up to 15x10^6 vc/mL (Figure 1), while an incremental increase in VVD should be applied to realize a high cell density as well as a high viability (Figure 2 and 3).
  - The TFDF filter has a sieving coefficient of around 1.0 for the enveloped vectors, since the filter size is more than 10 times the diameter of the vectors (Figure 1, 2 and 3).
- **Product Attributes:**
  - Although the physical titer in the permeate and retentate is gradually increased after perfusing for about 72 hours (Day 3) in the Mobius bioreactor, the infectivity of the vectors is significantly reduced (Figure 2). This could be due to heating of the particulates around the vessel walls and impellers after a loss of the cell culture volume to the permeate line.
  - The infectious titer in the permeate is significantly higher than that in the retentate in the Distek bioreactor (Figure 3), suggesting the vectors could maintain their infectivity after they are removed from the cell culture media at 37°C and stored at a lower temperature of 4°C.
  - A high turbidity of the collected permeate from both Mobius and Distek bioreactors (data not shown) requires an additional depth filter to remove the particulates in the permeate before it is applied to the purification column.
- **Fouling**
  - No fouling is seen for up to 10 days of perfusion processes (Figure 4), independent of various VVDs, with or without bleed, and the cell density.
- **Future work**
  - Address oxygen transfer inefficiency in both Mobius and Distek bioreactors when the cell density is above 50-60x10^6 vc/mL.
  - Apply continuous bleed when the VCD reaches around 30-40x10^6 vc/mL to prolong the vector production for more than 10 days with a consistent yield.
  - Optimize the bioreactor operation and conditional parameters, such as temperature, pH, oxygen uptake and feeding regimen, obtain consistent high infectivity of enveloped vectors in the permeate in both Mobius and Distek bioreactors.

CONCLUSIONS

Five perfusion runs have been conducted in bench-scale bioreactors with TFDF filters for producing the enveloped vectors. The viable cell density reaches up to 60 x10^6 vc/mL with incremental VVD values, while it is below 20 x10^6 vc/mL at a constant VVD value. The physical titer and infectious titer of enveloped vectors in the pooled permeate are very similar to those in the retentate after the first day of perfusion and equivalent titers for both in the permeate and retentate are seen for up to four days after perfusion. No fouling of the TFDF filters was observed during five runs, covering the cell culture duration up to 10 days. This proof-of-concept study demonstrates the feasibility and potential opportunity of applying TFDF filters in the perfusion process for enveloped vector production.

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