INTENSIFIED ENVELOPED VECTOR PRODUCTION FROM HEK293T CELLS IN FLEXSAFE® RM USING PERFUSION BIOPROCESSING

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ABSTRACT

To meet the escalating demands of biotherapeutics for the cell and gene therapy space, innovation in process technologies has become imperative. In recent years, continuous bioprocess technologies have gained popularity in part to greater productivity gained per facility footprint, while reducing time and cost of facility construction. Most importantly, the continuous removal of waste metabolites and replacement of fresh media helps maintain a stable cellular microenvironment, resulting in more consistent product quality profiles. In some cases, product degradation may be minimized by continuous product removal from the bioreactor culture environment.

This study evaluated the production of an enveloped vector derived from HEK293T cells in a bench-scale Sartorius rocker system equipped with a single-use Flexsafe@ RM perfusion bag with an integrated 1.2 µm filter membrane. Parameters such as pH, DO, and temperature, cell culture weight, rocking speed, and angle were controlled via a Biostat B2 Twin CC RM Rocker 50/50 platform. An external pump was used to feed fresh media into the perfusion culture, while a peristaltic pump on the control tower was used to remove cell-free product and spent media from the perfusion culture.

Our results demonstrate a perfusion process that extends the cell culture growth period beyond traditional batch and fed batch production modes. Notably, our findings reveal that this approach maintains a high cell viability > 95% and achieves viable cell densities > 80E06 VC/ML, resulting in a significant increase in vector production. These results also demonstrate the simplicity and capability of the Sartorius Flexsafe@ RM perfusion membrane bags as a perfusion platform for HEK293-based vector production.

INTRODUCTION

Enhancing bioproduction through a perfusion mode of production has revolutionized process development of biologics, enabling higher cell densities through the removal of spent media and toxic waste metabolites, while in parallel, providing continuous replacement of fresh media and vital nutrients to maintain cell proliferation. The synergy between higher cell densities and uninterrupted nutrient supply can lead to substantial increases in volumetric productivity.

Additional benefits include:

- Economics: reduced cost of goods from increased volumetric productivity; lower capital investment; smaller bioreactor and facility footprints; reduced bioreactor turnaround time; shortened production downtime and boosted flexibility for a diverse portfolio of products.
- Process and Product: improvement in product quality consistency via a pseudo steady state cell culture macroenvironment; maintenance of high cell viabilities; reduction of process impurities; and continuous harvest of labile products.

Technical challenges remain including:

- Optimization of the perfusion cell retention device to minimize the impact on enveloped vectors due to their fragile nature;
- Real-time analytical technologies for robust maintenance of optimal perfusion conditions;
- Adaption of current perfusion technologies, such as alternative tangential filtration (ATF), tangential flow depth filtration (TFDF) and tangential flow filtration (TFF) devices catering to monoclonal antibodies, for Cell and Gene Therapy applications.

In our previous report, stirred tank bioreactors are coupled with TFDF filters for production of enveloped vectors [1]. While the perfusion process can be extended to 6-8 days using the external filter, the operation had to be terminated due to a low viability in the stirred bioreactor. The aim of this study is to assess the feasibility of using Sartorius Flexsafe@ RM perfusion membrane bags with an integrated 1.2 µm perfusion membrane (PES) as an alternative of stirred tank bioreactors (STR) + TFDF to continuously harvest enveloped vectors. The membrane is fixed to the bottom of the bag. The membrane forms a barrier to retain cells inside the bag. The wave action of the cell culture fluid created by rocking allows flushing the membrane with every rocking motion. The permeate penetrating through the membrane is collected from the compartment below the membrane. In this study, cell growth, viability, and viral vector productivity were evaluated, and the functionality of the membrane bag was tested.

METHODOLOGY

HEK293T suspension cells were expanded in shake flasks prior to inoculation into the RM basic standard bag for the fed-batch (FB) process and Flexsafe@ RM bags for the perfusion process, and for the stirred tank bioreactors (STR at scale and 3L). The single-use bag was installed on a BIOSTAT® B2 Twin CC RM Rocker 50/50. After inflation of the bag and media batching, cells were inoculated at a working volume of 1L with different inoculation densities shown in Table 1. After saturation of the built-in optical pH and dissolved oxygen (DO) sensors installed inside the bag, online dissolved oxygen was controlled at 50%, and pH at 7.0. The temperature setpoint was 37 °C. The online pH was calibrated with offline pH measurements measured using a Bioprofile Flex Bioanalyzer. The physical titer and infectious titer of the vector of the retentate in the bag and the permeate were measured daily.

The fed-batch (FB) process starts with various inoculation density and let grow until a density saturation and or the death phase is observed before the culture is terminated. For the STR set-up, the bioreactor is connected to a DCU where the pH, temperature and dissolved oxygen are connected on a control loop [1].

The perfusion process started when the viable cell density reached about 4E06 VC/ML , and the vessel volume exchanged each day (VVD) was shown in Table 1. The permeate flow was controlled to ensure a constant rocker weight of 1.0 kg during the perfusion process. The rocking angle and speed were manipulated as the cell density increased (Table 1). A cell bleed was implemented to control the cell density from day 4 –10 and it was terminated for day 10 – 12.

Table 1. Experimental details for the perfusion processes.

Culture Process	Inoculation Viable Cell (iVC) Density (E06)	VVD	Peaked VCD (E06)	Harvest Period (Day)	Set Points: Temp. pH, DO	Rocking Angle, Speed,
Fed-Batch (FB) RM	0.5 - 1.5	NA	12- 15	2-3	37°C, 7.0±0.2, 50%	7-9^0, 25-34 rpm
Perfusion RM	0.5 – 5.0	0.3-3.5	40 - 80	1-12		7-9^0, 25-34 rpm
Perfusion STR[1]	1.5	0.5-3.5	25-55	1-11		190 rpm
FB STR	2.5	NA	12-15	2-3		CONFIDENTIAL

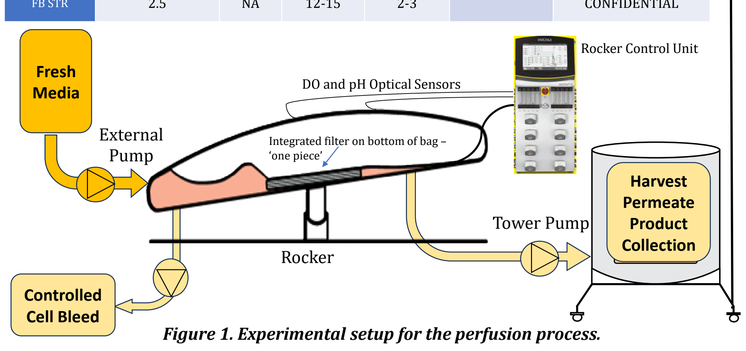

Figure 1. Experimental setup for the perfusion process.

RESULTS

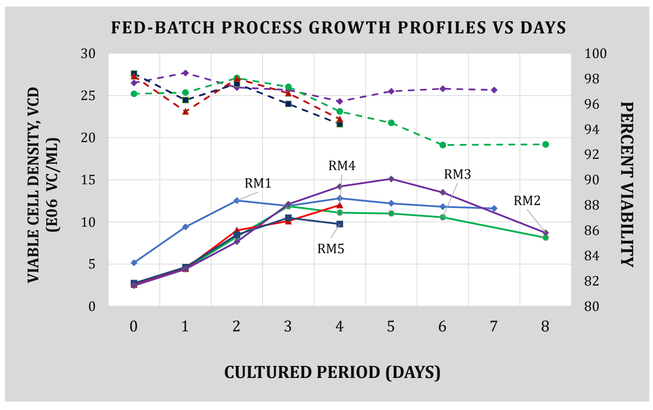

***Figure 1**. For the first set of RM bag experiments, three terminal fed-batch (FB) cultures (RM1-3) and two typical fed-batch production processes (RM4-5) were run. Typical growth profiles for HEK293 cell line were seen. All fed-batch cultures showed similar growth rates during the first 2-3 days in exponential phase, followed by 3–6 days of stationary phase, then followed by the death phase typically between day 6–7 of the cultured period. For the fed-batch production process, experiments were terminated within 3-4 days to harvest vector at peak titer, while maintaining a high cell viability of 95% or greater.*

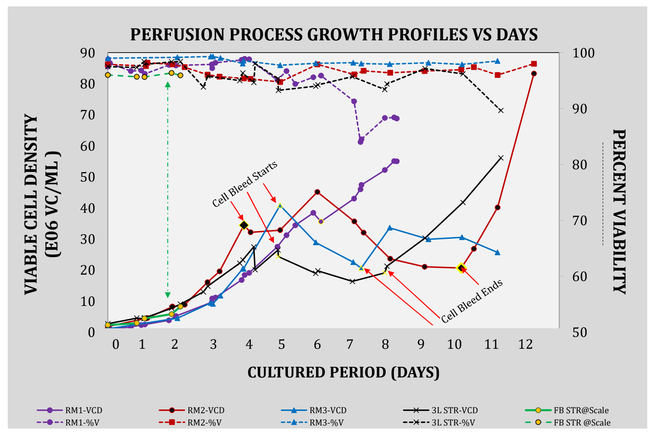

***Figure 2**. Several bench-scale perfusion runs (RM1-3) were performed utilizing the RM bags. While a typical stirred-tank (STR) fed-batch (FB) vector production run lasts 2-3 days for the most optimal vector harvest period; all bench-scale perfusion cultures were able to extend cell culture for additional growth phase up to 11-12 days, and maintained above 95% viability, when targeted cell bleeds was implemented. Perfusion runs also demonstrated the options for either continuous product collection through the permeate, or final product harvest accumulated in the retentate at a high cell density.*

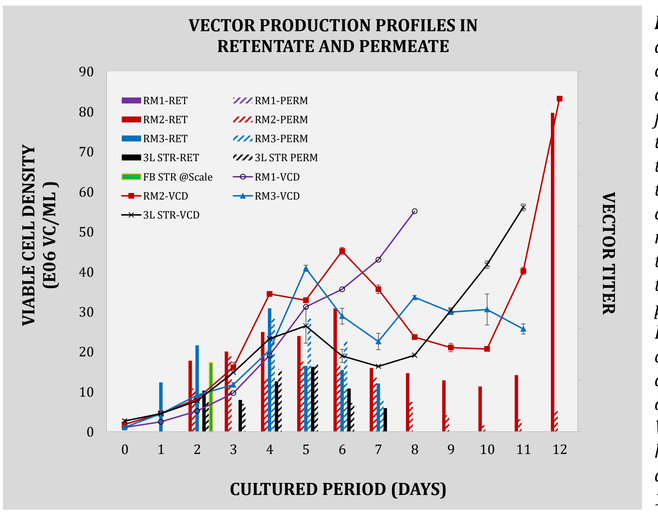

***Figure 3.** Vector production in the retentate and permeate in the RM bag. There was a correlation between vector titer production and the cell viable density (VCD) in the RM bag for the first 6-7 days. The vector permeated through the internal membrane and the vector titer in the permeate was equivalent to that in the retentate. In the RM2 run, the vector collected in the permeate through the membrane was seen for up to 12 days, while the vector titer in the permeate was lower than that in the retentate, due to possible partial blockage of the membrane [2,3]. Perfusion mode of operation allows for the cells in the exponential growth phase to reach a particular harvest target or maintain at an optimal cell density for impurity management. When compared to one 2-day fed-batch vector harvest (green bar), the perfusion culture allowed for continuous vector harvest for up to 12-day period.*

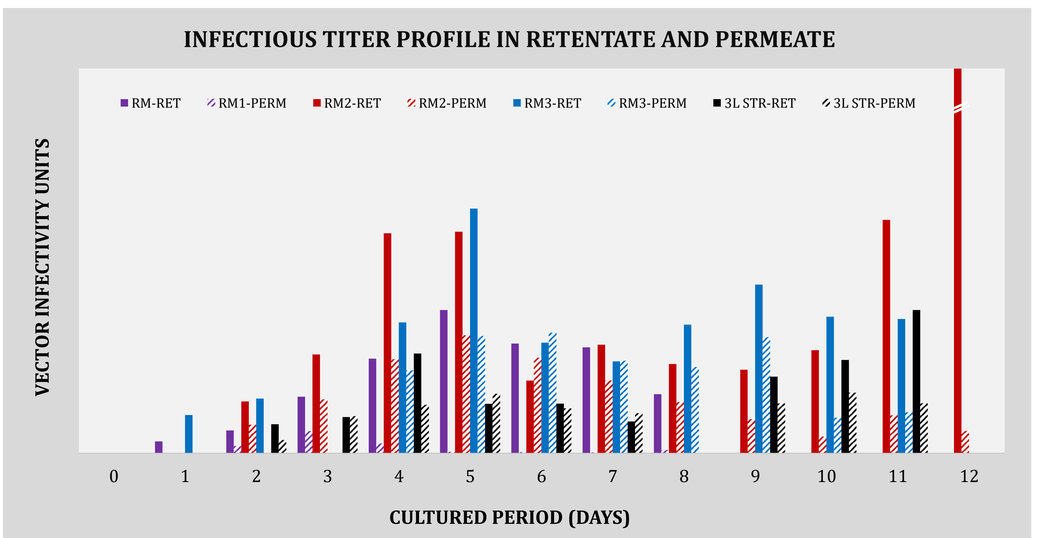

***Figure 4 .** Infectious titer in the retentate and permeate for three perfusion RM bag runs is represented in the figure above. The infectivity titer in both permeate and retentate initially increased as cell density also increased (day 1-5) and continued at a stable production rate after implementation of the cell bleed (day 6-9). The infectivity of the vector is critical for transducing the cells; therefore, it is extremely important to maintain the vector infectivity during the production phase. Typically, the half-life of the vector at 37°C is around 8 hours, for a batch and fed-batch process, the vector remains inside the bioreactor the entire cultured period, usually lasting for up to three days (> 72 hours), thus destroying some of the earlier vector production [4]. But, with the perfusion operation, this allows continuous vector harvest preserving its infectivity. In the early cultured period within 6-7 days, the infectivity units collected in the permeate was equal to those remaining in the retentate (solid bar vs striped bars), suggesting removing the vectors during the perfusion process helps protect the vector infectivity. As the culture was extended longer than 7 days, the infectivity units in the permeate was less than infectivity units in the retentate, which was consistent with the observations of partial perfusion membrane blockage shown in Figure 3.*

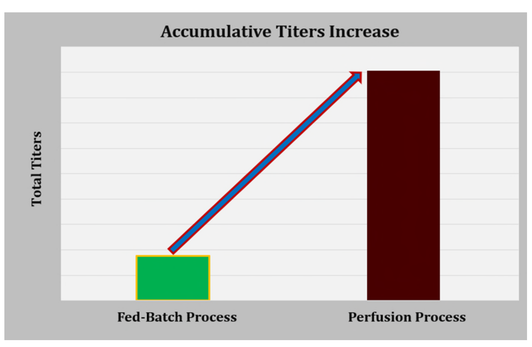

***Figure 5**. Accumulative titers during a perfusion process compared to the titer from a typical fed-batch process. After integration of the infectious titer over the harvested permeate volume, the total infectious titer was normalized to the bioreactor volume for a fed-batch process. Comparing the total titer from one perfusion RM bag process to one harvest from a fed-batch process in a stirred tank, the cumulative vector production shows significant fold increase in product throughput.*

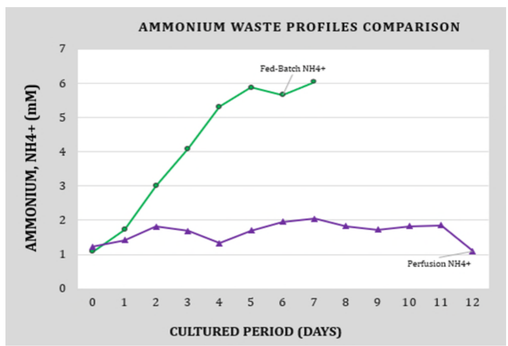

***Figure 6**. Representative ammonium trends for a terminal fed-batch RM bag culture and a perfusion RM bag culture. After perfusion was initiated in the perfusion RM bag, ammonium levels were maintained under 2 mM while the terminal fed-batch culture ammonium level peaks around 6 mM. The fed-batch culture most likely entered stationary growth phase between days 4-5 due to waste metabolite accumulation. The perfusion process continuously removed waste metabolites and maintained waste accumulation below a certain level, which allowed cells to remain in the exponential growth phase up to day 12 of the perfusion culture, as can be seen in Figure 2.*

DISCUSSION

Fed-Batch (FB) versus Perfusion Process Growth Profiles:

- In the fed-batch process, the exponential phase is limited to 2-3 days, with a Peak VCD around 10-11E06 VC/ML [Figure 1]. The waste metabolites accumulated in the bag maybe the reason for the reduced cell growth rate and cell viability after the cells reach stationary phase [Figure 6].
- During the perfusion process, the exponential phase is extended to a longer period of up to 12 days, and could be further prolonged, when cell bleed is implemented, as shown in Figure 2. Since the waste metabolites are continuously removed from the bag and the culture environment is maintained at a lower toxic level [Figure 6], a favorable and stable environment could be maintained for a longer period of vector production.

Vector Production Profiles:

- Fed-batch process: The vector is typically harvested between day 2-3, when the cell viability is greater than 90%, therefore limiting the viable cell density to between 8-12E6 VC/ML and forcing a one-time harvest that can significantly limit vector productivity when compared to a perfusion mode of operation [Figure 3].
- Perfusion process: The perfusion mode allows for continuous and immediate harvest of the vector for up to 12 days in the RM bag [Figure 3], where the vector infectivity in the permeate is preserved during continuous collection of the product to a container that can be held at lower temperatures to preserve the infectivity [Figure 4]. The accumulative titer from continuous harvest achieves a greater fold increase within one perfusion run and may be equivalent to several fed-batch runs [Figure 5].

Infectious and Accumulative Titer Profiles:

- Preserving the infectious titer of the vector during the upstream production is of major importance, however, it can also be very challenging due to possible filter blockage from cell debris or particulates.
- Figure 4 represents the challenge of harvesting all the vector produced. After the internal filter blockage, the amount of the vector collected in the permeate is significantly reduced after day 6-7, while most of the infectious vector is retained in the RM bioreactor instead of being harvested in the permeate.
- Ideally, the optimal collection period can be prolonged to 7 days, resulting in a higher folder increase than what the fed-batch process would be able to produce [Figure 5].

CONCLUSION

The results from the study show that a continuous perfusion culture with a high cell density can be achieved over a longer period than a comparative fed-batch process, while maintaining cell viability greater than 90% and increasing the VCD ranging from 50-80E06 VC/ML.

The longer perfusion production period allows for multiple harvests in one run versus a one-time harvest for one fed-batch process. Meanwhile, removing toxic metabolites that may hinder cell growth and continuously harvesting the product to preserve its infectivity can reduce the residence time of a product within a bioreactor, which can lead to more desirable product quality.

The advantage of perfusion manufacturing is the capability of continuous harvest and accumulative higher levels of productivity over fed-batch systems, thereby reducing production costs and offers a quicker timeline to manufacturing campaigns.

The RM bag with an internal membrane filter is easily implementable for initial perfusion process development. The RM bags are equipped with optical pH and DO sensors that are similar to stirred-tank bioreactors. However, the filter fouling is challenging to monitor without pressure sensors in the RM bag and generally means careful monitoring of rocker culture weight.

REFERENCES

1. Svay, L., Zhang, H., and Johnson, R.G. (2023). Intensified enveloped vector production using continuous perfusion processing. The 26th ASGCT Annual Meeting.
2. Pollock, J., Coffman, J., Ho, S. V., & Farid, S. S. (2017). Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. Biotechnology Progress, 33(4), 854–866.
3. Tran, M.Y. and Kamen A.A. (2022). Production of Lentiviral Vectors Using a HEK-293 Producer Cell Line and Advanced Perfusion Processing. Front. Bioeng. Biotechnol, 10:887716.
4. Voisard, D. 2003. Potential of Cell Retention Techniques for Large-Scale High-Density Perfusion Culture of Suspended Mammalian Cells. Biotechnol. Bioeng. 82:751-765.

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GenVivo Inc. is a clinical-stage gene therapy company headquartered in San Marino (Pasadena), CA, currently advancing a cancer immunotherapy enveloped vector product candidate.