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Innovative Horizons: Perfusion-driven Process Intensification for HEK293 cells in Cell and Gene Therapy

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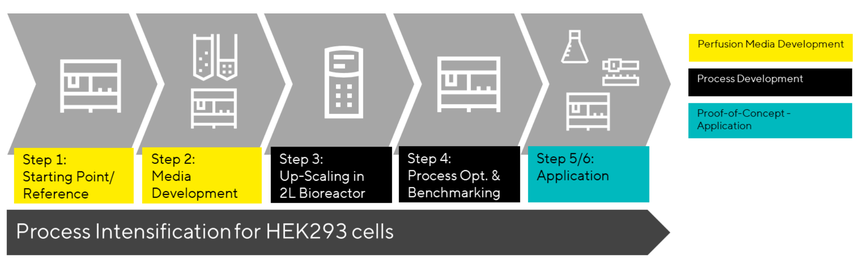
Introduction

The rapid advancement of cell and gene therapies opened new avenues for treating diseases once considered incurable. Despite this progress, high cost and limited availability of these therapies present significant challenges, necessitating solutions to meet the growing demand from eligible patients. While perfusion-based process intensification techniques have been the standard in protein-based therapies produced in CHO cells for years, they remain a novel and expanding field for HEK293 cells. The use of HEK293 cells offers the advantage of producing not only proteins but also various viral vectors, such as Adeno-Associated Virus (AAV), Lentivirus (LV) and Adenovirus (AdV).

1. Objective

The objective was to develop an innovative cell culture medium specifically for perfusion processes with HEK293 cells, designed to support high cell densities by providing sufficient nutrients. This medium aims to enhance the N-1 step in transient processes, such as most AAV processes, or enable production at high cell densities using stable HEK293 cell lines, for instance for AdV or LV manufacturing.



2. Development of a Perfusion Medium

To create a culture medium that meets the specific requirements of a perfusion medium, the initial formulation HEKlean P1 was developed combining rational design and statistical methodologies including Design of Experiments (DoE). HEKlean P1 was evaluated in a perfusion process using the Ambr®250 (Sartorius) (Fig. 1). Peak cell densities of approx. 55E6 cells/mL were achieved at 2 Vessel Volumes per Day (VVD) and with a stable bleed from day 3 onwards. To further optimize the medium for perfusion, combinations of several compounds were added during steady state at 20E6 cells/mL in the Ambr®250 (Fig. 2). Deviations from the target cell density caused by increased cell growth led to identification of several components that are beneficial for HEK293 cultivation in perfusion processes.

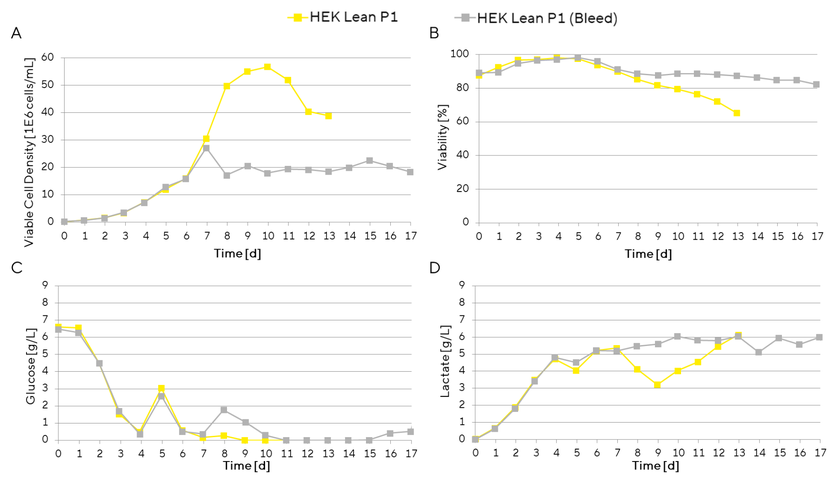


Figure 1: Cultivation of HEK293 cells in a regulated system (Ambr ®250 , Sartorius) in a continuous process with no bleed and bleed at 20E6 cells/ml with different VVDs over cultivation time course. HEK Lean P1 can sustain VCD of 55E6 cells/mL at 2 VVD (A) at 81 % viability (B) at maximum cell density on day 10. Despite increasing VVD and glucose feeding, glucose is depleted shortly afterwards (C) and lactate tends to accumulate to high levels despite 2 VVD (D).

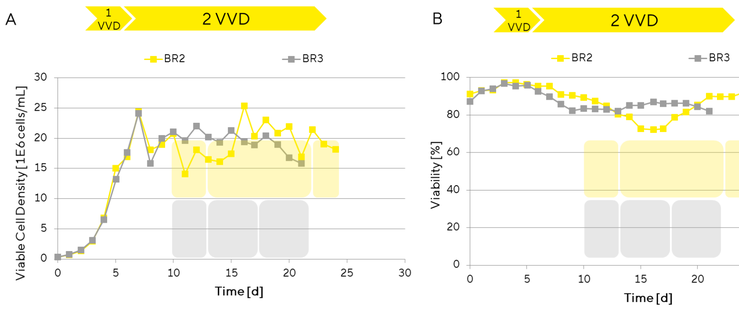


Figure 2: Steady-state during perfusion to test different media variants. Using HEKlean P1 at indicated VVDs, HEK293 cells were cultivated in a steady state at 20E6 cells/mL in the Ambr®250 (Sartorius). In phases indicated by the boxes, individual compounds or combinations thereof were added to the culture medium. Beneficial or detrimental effects were identified by positive or negative deviation from the target cell density, respectively.

3. Process Development

To define a new medium formulation for perfusion, process development was conducted in parallel. The HEK293 perfusion process was transferred from the Ambr®250 to a 2 L Biostat® B Twin (Sartorius) combined with an Xcell ATF® system (Repligen). By switching from variant P1 to P2, VVD, and bleeding, a peak cell density of approx. 80E6 cells/mL was achieved, and the perfusion process was successfully maintained for up to 31 days (Fig. 3). New media variants were developed based on spent media analytics.

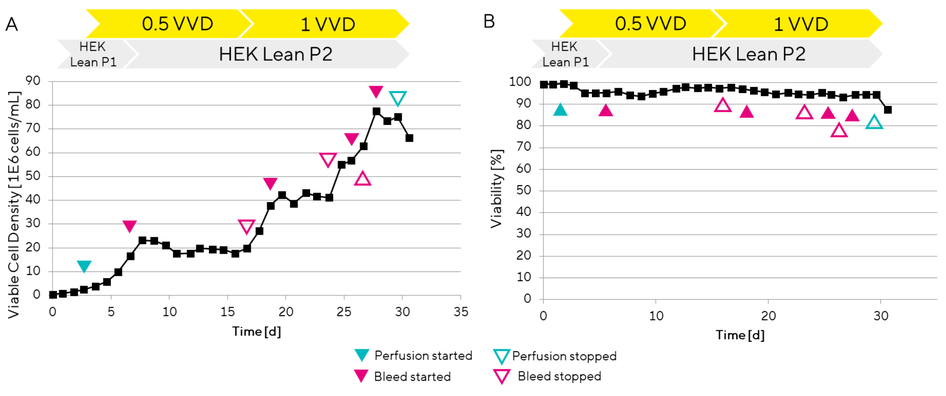


Figure 3: Upscaling of perfusion process for HEK293 cells in 2 L benchtop bioreactor Biostat® B Twin (Sartorius) with Xcell ATF® system (Repligen) up to 31 days. Perfusion started on day 3 without additional glucose feeding. Using media variant HEK Lean P2, cell densities of approximately 80 million cells/mL could be achieved (A) with high viabilities throughout the whole process at low VVDs (B).

4. Process Optimization and Benchmarking

New media variants were benchmarked against a perfusion process with HEK 293T cells described in literature [1] using the established process in the Ambr®250 (Sartorius). HEK293 cells were cultivated over a 16-day period at 1 VVD in three distinct HEK Lean media variants – HEK Lean P2, P3.1, and P3.7 - alongside a competitor medium. For further process intensification, a nutrient-rich HEK Lean P3.6 variant was tested at 0.7 VVD. With HEK Lean P3.7, approx. 100E6 cells/mL were achieved while maintaining a viability of approx. 90 %. Thereby, the formulation outperformed all other variants as well as the competitor product.

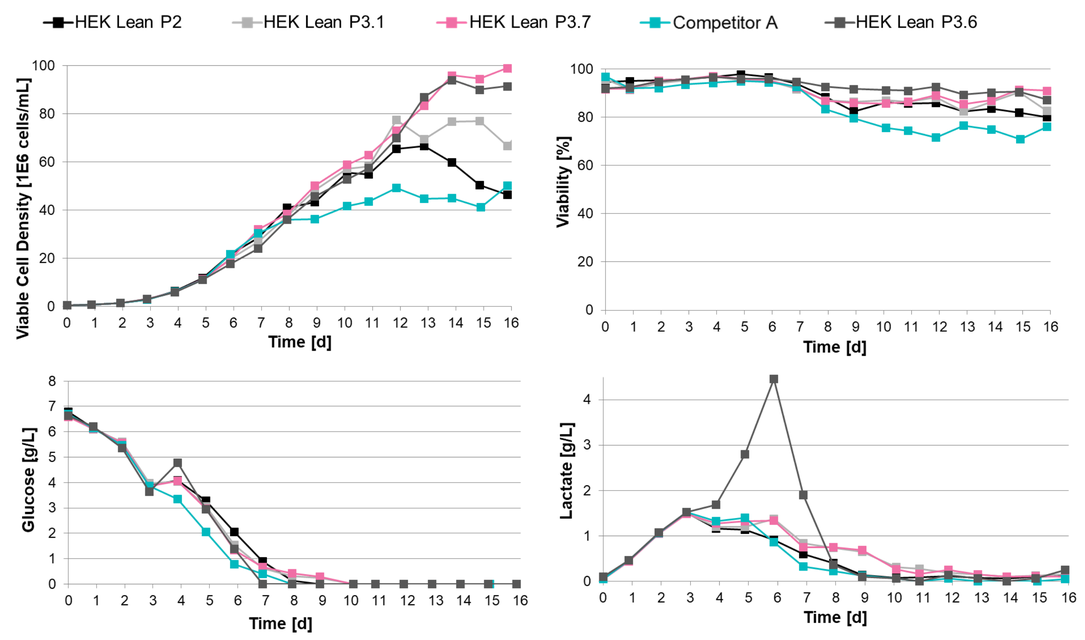


Figure 4: Cultivation of HEK293 cells in a controlled system (Ambr®250, Sartorius) in a continuous perfusion process in four different HEK Lean media variants and one competitor medium selected based on literature [1]. Dissolved Oxygen (DO) was set at 40 % and pH at 7.05 ± 0.05. P2, P3.1, and P3.7, alongside a competitor medium, were cultivated over a 16-day period at 1 VVD, whereas HEK Lean P3.7 was tested with reduced VVD at 0.7. HEK Lean P3.6 and P3.7 achieved > 90E6 cells/ml (A) on day 14 at high viabilities > 80 % (B). After start of perfusion on day 3, lactate tends to accumulate to higher levels in HEK Lean P3.6 until day 6 (D).

5. Application for N-1 step – Transient AAV Production

To test the developed medium for use in an N-1 step in transient AAV production, a bleed bioreactor (Ambr®250, Sartorius) with 20E6 cells/mL was used. HEK293 cells were transfected at 3E6 cells/mL following the recommended protocol for FectoVIR®-AAV (Sartorius). Moreover, the impact of glucose feeding on AAV production was evaluated in this setup. As a reference, a culture grown in shake flask instead of Ambr®250 perfusion was used. Importantly, cultures inoculated from an N-1 bleed bioreactor yielded similar genomic and higher transducing titer compared to standard transfection processes.

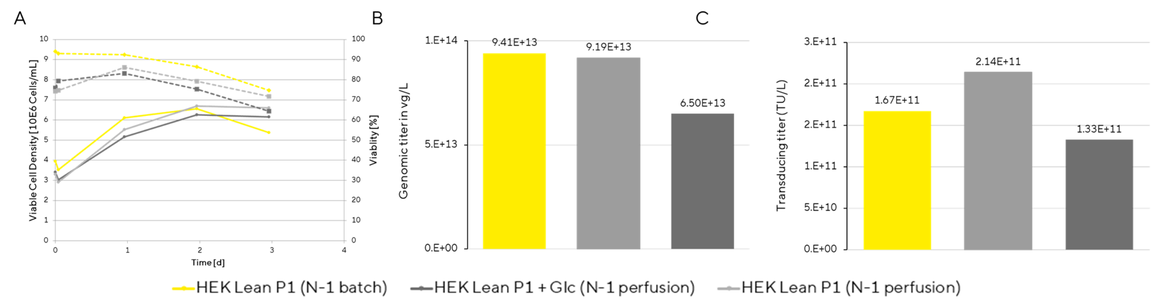


Figure 5: Proof-of-concept for application in N-1 step for transient AAV production using a bleed bioreactor at 20E6 cells/mL in comparison to inoculation from shake flask. For AAV-2 production, HEK293 cells were transfected with FectoVIR®-AAV (Sartorius) in HEK Lean P1. Viable cell densities and viabilities were monitored throughout the process with comparable progressions between the different conditions [A]. Genomic titer was assessed by ddPCR [B] and transducing titer on adherent HEK cells using Incucyte® (Sartorius)[C].

6. Application for stable production - AdV Infection

To test the suitability of the media for other modalities, the HEK Lean P3.7 medium was used in a batch cultivation for adenoviral infection. Inoculation was done at 0.5E6 cells/mL and infection at 4E6 cells/mL with 5 multiplicity of infection (MOI) on day 3 in Ambr®15 (Sartorius). At 48 hours post infection, the capsid titer was quantified using Octet® (Sartorius). Best cell growth was achieved using the competitor media (Fig. 6A), while highest capsid titer were reached using HEK Lean P3.7 (Fig. 6B).

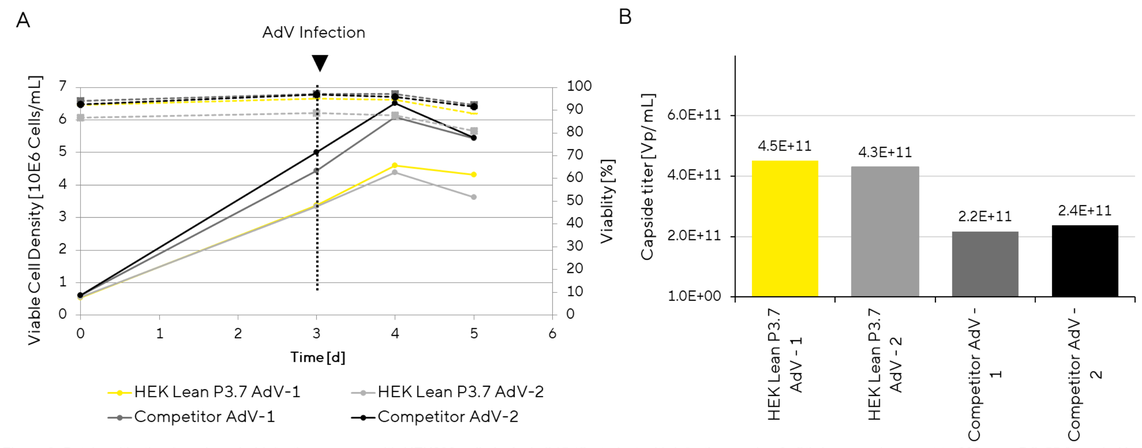


Figure 6: Batch cultivation for adenoviral infection process with HEK293 cells in Ambr®15 (Sartorius) with infection on day 3. Stirring speed was set to 600 rpm, DO 55 % and pH at 7.25 +/-0.05. Viable cell densities and viabilities were determined using integrated cell counter (A). The capsid titer was quantified using Octet® (Sartorius) 48 hours after the infection (B).

Conclusion

In summary, the results of this study indicates, that

- ☑ VBT application can benefit from process intensification
- ☑ developed HEK Lean media variants are suitable for N-1 perfusion for AAV production
- ☑ developed media can be used for stable producer processes, here AdV infection

[1] JP Mendes et al. (2022) AAV process intensification by perfusion bioreaction and integrated clarification, Frontiers in Bioengineering and Biotechnology, 10.3389/fbioe.2022.1020174