Efficient HEK cultivation with small-scale single-use bioreactor

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State of the art

HEK293 cells are popular for biotechnological and therapeutic applications, contributing to FDA-approved gene therapies since 2015 [1–6].

HEK293 cells can be successfully cultivated in suspension using stirred tank bioreactors [7–10], which offer scalability and reproducibility. In addition to this, single-use bioreactors reduce the risk of cross-contamination.

HEK293 are cultivated using different operating modes, while batch mode is common due to simplicity and cost, fed-batch strategies have been shown to improve viable cell density (VCD) and viability (%) [8].

Aim

The aim of this study was to evaluate the suitability of the small-scale single-use bioreactors for the cultivation of HEK suspension cells using batch and fed-batch (bolus addition of efficient feed) as modes of operation.

Materials and methods

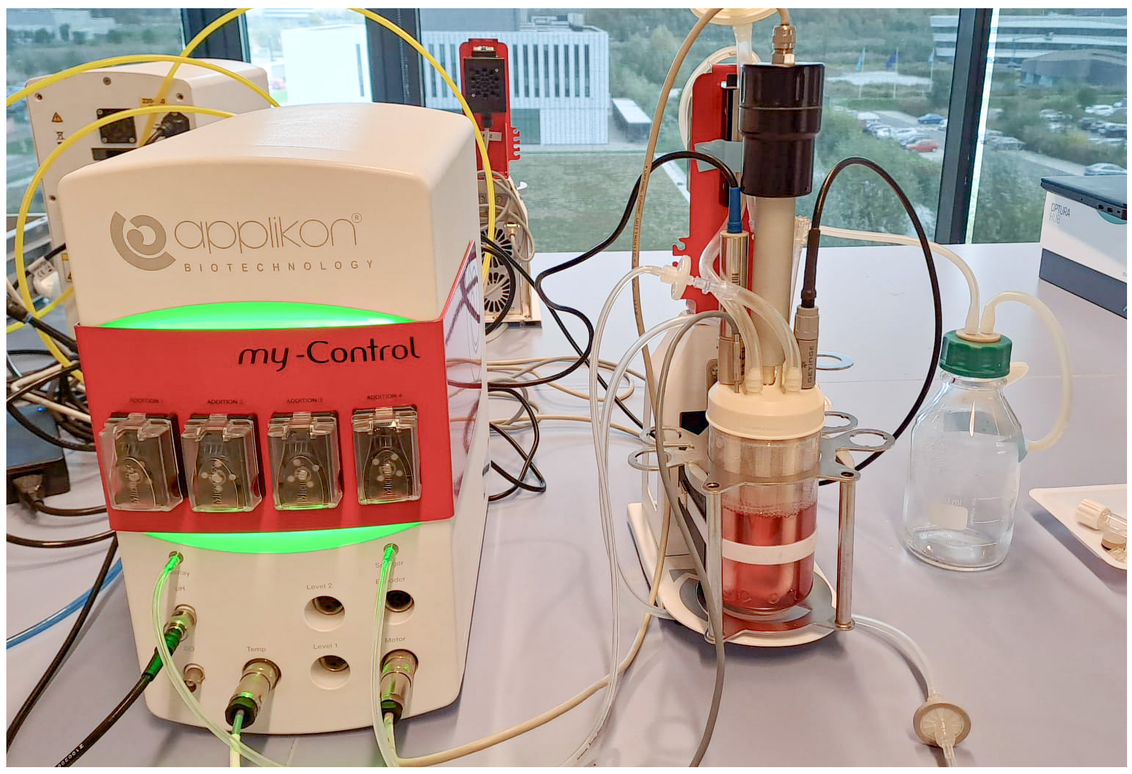


Figure 1 | Setup of the my-Control biocontroller with the AppliFlex ST 500 mL.

Cell Line and Media

Expi293F™ cells (Fisher Scientific) were cultured in Expi293™ Expression Medium (Gibco™). Pre-cultures started from a cryopreserved cell bank stored in liquid nitrogen with 10% DMSO.

Shake Flask Pre-Culture

Cells were maintained in 125 mL filter-capped shake flasks with a 30 mL working volume. Cultures were passaged every 3–4 days, starting at a density of 0.4–0.5 × 10^6 cells/mL to ~4 × 10^6 cells/mL. Incubation conditions: 37˚C, 130 rpm, 8% CO_2, and 85% relative humidity (supplier instructions). Cultures were not used beyond 20 passages.

AppliFlex ST Bioreactors

The single-use bioreactors used for this study can be supplied for two different applications, research and development (R&D) and clinical production (GMP). The dimensions, configurations and materials are the same for both types. The difference lies within a post treatment on a contact material, the qualification documentation and production requirements, where the GMP version is supplied to meet all requirements for clinical applications. To verify the performance and no need for technology transfer between the R&D and GMP model, Fed-batch 3 experiment was run with the GMP version. The other experiments shown in this poster were performed with R&D versions.

Bioreactor Setup and Culture Conditions

Batch and fed-batch experiments were conducted in a 500 mL AppliFlex ST single-use bioreactor (working volume: 250 mL), equipped with a reusable LumiSens optical DO sensor and an AppliSens pH sensor, controlled via the Applikon my-Control system (Figure 1). A tip speed of 0.66 m/s was used for agitation (450 rpm in this case).

- Dissolved Oxygen (DO) was maintained at 40% air saturation via pure oxygen sparging through an open pipe sparger.
- pH was controlled at 7.1 by sparging CO_2, with the headspace continuously flushed with air to remove excess CO_2.
- The bioreactor was inoculated at 0.4 × 10^6 cells/mL when the batch was used as mode of operation, as done previously in shake flasks during passage and maintenance.
- The bioreactor was inoculated at 0.65 × 10^6 cells/mL during the Fed-batch 1 run. The inoculum concentration was 1.0 × 10^6 cells/mL in Fed-batch 2 and Fed-batch 3. In these experiments, at mid-exponential phase (2-2.5 × 10^6 cells/mL), Efficient-Pro™ Feed 2 was added in bolus doses of 0.4% (v/v) every 24 hours using the integrated pump of the my-Control system, as recommended by the media supplier.

Sampling and Analysis

Duplicate daily samples were collected for:
- Viable Cell Density (VCD) and viability, determined using a hemocytometer and trypan blue staining.
- Metabolite analysis (Glucose, Lactate, Glutamine, Glutamate), conducted on a YSI biochemical analyzer at Wageningen University. Supernatants were stored at –20˚C prior to analysis. These analyses took place for Batch 3, Fed-batch 2 and Fed-batch 3.

Results

Batch cultivation

HEK293 cells were grown in the AppliFlex ST 500 mL bioreactor using batch mode (Figure 2). The three replicates showed high reproducibility, with an average maximum VCD of ~6.5 × 10^6 cells/mL. This peak occurred when glucose and glutamine were depleted, and lactate concentration also reached its maximum.

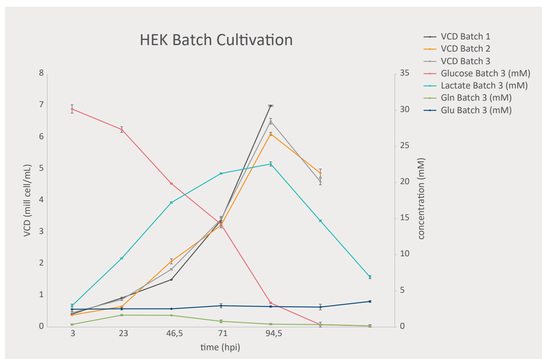
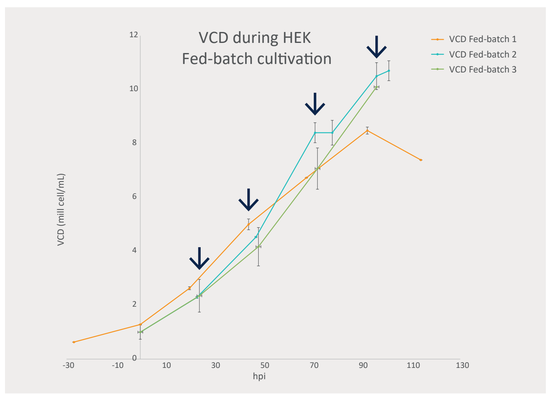


Figure 2 | Viable cell density (VCD) of three replicates of batch cultivation and glucose, lactate, glutamine and glutamate concentration profiles of the Batch 3 experiment.

Fed-batch cultivation

Figure 3 illustrates the VCD of three fed-batch runs and the glucose, lactate, glutamine, and glutamate concentration profiles of two runs. The VCD of the 3 runs show the same profile. Glucose and glutamine were nearly depleted by 80 hours post-inoculation, followed by lactate consumption, indicating a metabolic shift. Glutamate dynamics remained stable, consistent with cell metabolic demands. Daily bolus additions of Efficient-Pro™ Feed 2 are marked by blue arrows. Fed-batch cultivations reached a maximum average VCD of ~9 × 10^6 cells/mL, compared to ~6.5 × 10^6 cells/mL in batch-mode cultivation (7-9). This indicates that further improvement is possible by increasing the feed amount towards the end.



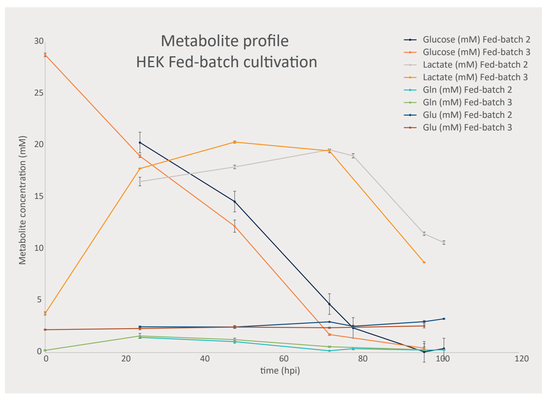


Figure 3 | Viable cell density (VCD) of three fed-batch runs and glucose, lactate, glutamine and glutamate concentration profiles of the Fed-batch 2 and 3 experiments. The blue arrows indicate the bolus addition.

Viability

Cell viability remained between 94-100% throughout the 100-hour run, showing moderately better results in the fed-batch cases. These results align with previous studies, confirming the benefits of the fed-batch operation.

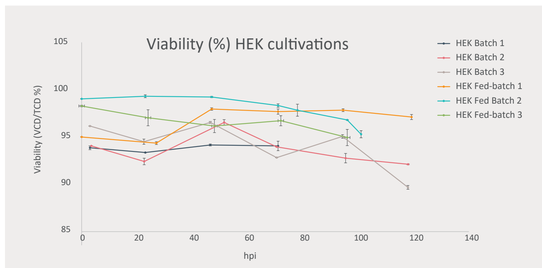


Figure 4 | Viability (%) profile of the six runs.

pH and Lactate Correlation

Figure 5 shows the relationship between extracellular lactate concentrations and pH over time. The pH profiles of Fed-batch 2 and Fed-batch 3 runs are compared to lactate concentration profiles. After 72 hours post-inoculation, lactate concentration decreased due to late-stage culture consumption, coinciding with a rise in pH, as reported in HEK293 cultures (11-12).

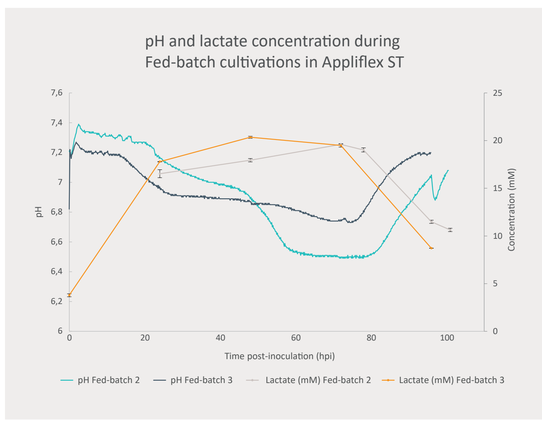


Figure 5 | pH and extracellular lactate concentration profiles during two replicate HEK293 cultivations in the AppliFlex ST bioreactor.

Conclusions

The results show that HEK293 cells can be successfully cultivated in the AppliFlex ST bioreactor. The fed-batch strategy significantly improves viable cell density, outperforming batch mode. Metabolite trends (glucose and glutamine depletion, lactate production and consumption) align with published data, confirming the systems reliability and reproducibility. Overall, the small-scale AppliFlex ST bioreactor is effective for HEK cell cultivation, scalable process development and high-throughput experimental studies. These findings validate its suitability for upstream process optimization in a controlled, miniaturized setting. Future experiments could aim to optimize bolus addition to further improve VCD and explore other pH control strategies like two-side control.

References

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