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Optimization of the HEK293T Suspension Cultivation With a DOE-Approach in Ambr® 15 Cell Culture

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Abstract

The automated and high throughput properties of the Ambr® 15 Cell Culture system, with MODDE® software, were used to design experiments and reliably screen the critical process parameters, through parallel processing at microscale.

In the study presented we used Ambr® 15 Cell Culture for the optimization of HEK293T culture in suspension. We identified optimal stirring speed, dissolved oxygen (DO) and pH value by performing a DOE study with the use of MODDE® software for experimental planning. We observed that cultivation of HEK293T cells in the Ambr® 15 microbioreactor yields improved cell growth and viability as compared to standard shake flask culture. We identified that pH was the most significant factor - besides stirring speed - which has a lesser significant impact on cell health and growth.

This study demonstrates that the Ambr® 15 microbioreactor system in combination with the DOE software MODDE® enables a systematic investigation of critical process parameters and rapid, high throughput process improvement and optimization. The results prove that the transition from shake flask to a scalable stirred bioreactor system can be accomplished in a timely manner.

Find out more at:
www.sartorius.com/en/products/fermentation-bioreactors/ambr-multi-parallel-bioreactors/ambr-15-cell-culture

Introduction

In viral vector production processes for cell and gene therapy applications, HEK293T cells are are usually transfected with the genetic nformation of the virus. However, new stable cell lines now exist that produce the viral vector themselves [1; 2].

Currently, viral vectors are often produced in adherent culture processes, for example, in cell factories or cell stacks. However, virus production in these formats is difficult to handle and limited in scalability. The transition to a 3D cultivation in suspension in a bioreactor could be an option to solve these issues. Therefore, a significant challenge for the cell and gene therapy industry is to develop HEK293T suspension cell culture processes in a bioreactor, that are well characterized and can be scaled up for production, to ensure clinical and commercial success [3].

Ambr® 15 Cell Culture is an automated microscale bioreactor system that mimics the features and process control provided by much larger scale bioreactors, in a volume of 10 - 15 mL. The system offers parallel processing capability and excellent reproducibility which enable rapid, high throughput process improvement and optimization, including Design of Experiment (DOE) studies.

High throughput tools with parallel processing capabilities, such as Ambr® 15, help to address a major manufacturing bottleneck. They can be used as scale-down models for critical process parameter (CPP) screening [4], clone selection and effective media optimization in less time, with reduced reagent use and labor savings [5].

Design of Experiments, is a rational and cost-effective approach to practical experimentation that allows the effect of variables to be assessed, using only minimal resources. The MODDE® software package for DOE studies, enables fast and effective identification of critical process parameters and, subsequently, establishment of a design space, resulting in reduced bioprocess complexity and increased process understanding [6].

In this study, we describe how we used the Ambr® 15 Cell Culture system, for the optimization of HEK293T suspension cultivation. We identified optimal stirrer speed, DO and pH value, using a DOE approach with MODDE® software for experiment planning. Viable cell count and viability were monitored and compared to those in standard shake flask culture.

Abbreviations

CAR	Chimeric Antigen Receptor
CPP	Critical Process Parameter
DO	Dissolved Oxygen
DOE	Design of Experiment
RPM	Revolutions Per Minute
STR	Stirred Tank Reactor
VCC	Viable Cell Count

Figure 1

Ambr® 15 Cell Culture Generation 2

Materials and Methods

Adherent HEK293T (ATCC) cells have been adapted to suspension culture under serum-free conditions. The optimal culture medium was CD293 + 4 mM Glutamax (Gibco). Before starting the experiment, the cells were thawed and passaged at least twice.

In both experiments, a standard shake flask was used to culture cells, and the results analyzed as a reference control, in parallel to the cultivation in Ambr® 15 Cell Culture. Cells were seeded at $3x10^5$ c/mL and cultivated at 120 rpm stirring speed and 8 % CO_2 in a 125 mL baffled shake flask.

The first experiment was set up to identify the optimal setpoints of three process parameters, using the Ambr® 15 Cell Culture. Stirring speeds were investigated in a range of 400 to 800 rpm, pH between 6.8 and 7.4 and the percentage of DO between 30 and 70. In the second experiment, a lower stirring speed of 300 rpm was tested against the same speed of 400 rpm. The effect of the pH on cell growth was again investigated, with pH in a range of 7.1 to 7.4, with DO always at 50%.

The responses monitored were viable cell count and viability, which are typical readouts for HEK293T and viral vector production processes [1; 2]. The sparged vessel type was used for gassing with a culture volume of 15 mL (Figure 2). The cells were seeded at a density of $3x10^5$ c/mL. Daily antifoam addition was necessary to prevent foaming. The cultivation was performed for 5 days.

The integrated simplex (Nelder-Mead method) based optimizer routine in MODDE® was used to identify simultaneously set-points for all factors that fulfilled the specification for the selected responses. The optimization considered all responses and used the DOE models generated to interpolate in between experiments performed.

Figure 2

Ambr® 15 Cell Culture Sparged Vessel

Experimental Design

Experiments 1 and 2

Setpoints - Ambr® 15 Cell Culture using sparged vessels:
- bioreactor temperature: 37° C
- inoculation cell density: $3x10^5$ cells/mL
- fill volume: 15 mL, inoculum volume: 2 mL
- daily antifoam c addition (20 µl of 2 % solution)

Setpoints - shake flask:
- incubator temperature: 37° C
- inoculation cell density: $3x10^5$ cells/mL
- shaking rate: 120 rpm (baffled flask)
- fill volume: 37.5 mL
- CO_2: 8 %
- orbit: 5 cm

A two level full factorial design, with three centerpoints was used in setting up the DOE with MODDE® software (Experiment 1).

Table 1

Overview of Process Parameters, Readouts and Design of the DOE Study

Process Parameters	Range	
Stir speed (rpm)	400	800
pH	6.9	7.3
DO (%)	30	70
Responses	VCC, viability	

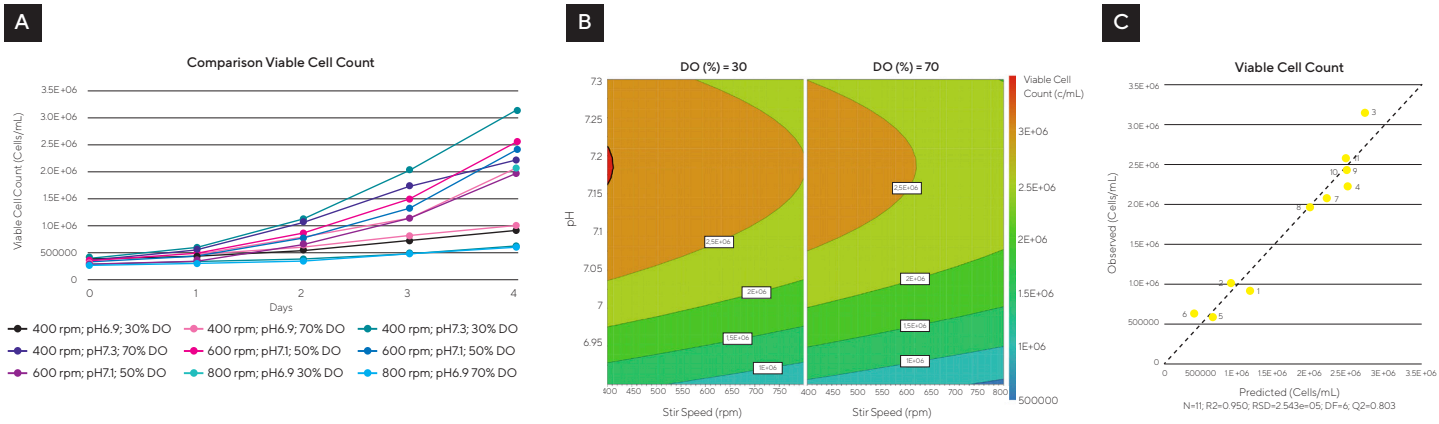
Bioreactor No.	DO	pH	Stir speed
1	30	6.9	400
2	70	6.9	400
3	30	7.3	400
4	70	7.3	400
5	30	6.9	800
6	70	6.9	800
7	30	7.3	800
8	70	7.3	800
9	50	7.1	600
10	50	7.1	600
11	50	7.1	600

Results and Discussion

Experiment 1

The aim of the first experiment was to identify optimum stirring speed, DO and pH value for the cultivation of suspension-adapted HEK293T cells in the Ambr® 15 Cell Culture by using a DOE approach.

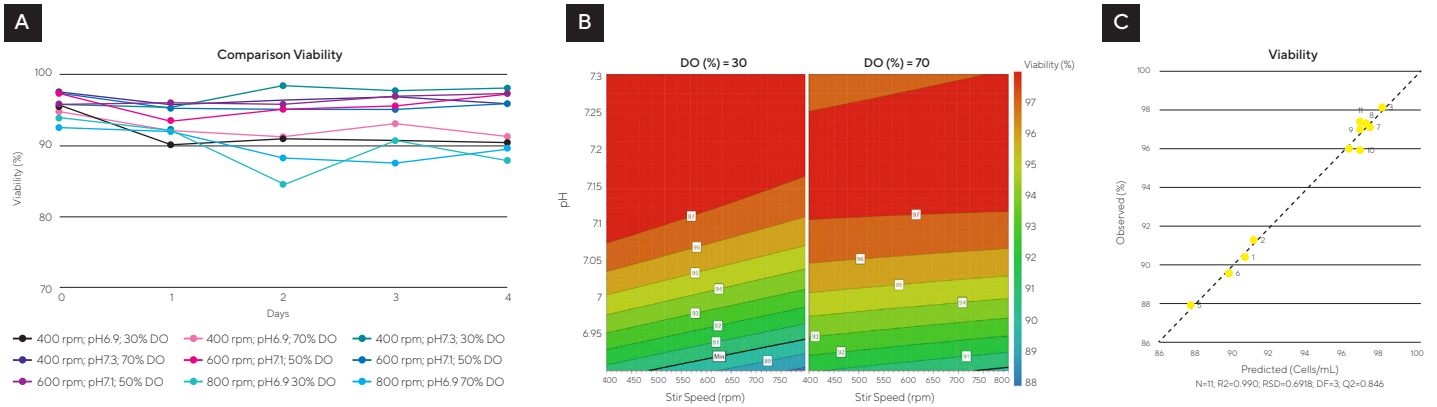
Figure 3



Note. A: Comparison of the viable cell count of the cells grown in the Ambr® 15 Cell Culture under different cultivation conditions. Each line represents a different condition, as determined through the DOE. B: Response contour plot of the viable cell count in relation to pH, DO and stirring speed. C: Observed-versus-predicted plot.

Variable cell densities were achieved which depended on the cultivation conditions in the vessel (Figure 3 A). Data has been compiled in a response countour plot to facilitate analysis (Figure 3 B). High cell counts are colored in red and low in blue. At conditions with a lower stirring speed around 400 rpm and at a pH value around 7.2, the cell count increased, as can be seen by its orange | red colour. We also found the validity of our DOE model to be very good, higher than 0.25. When analyzing the DOE for the parameter VCC with MODDE®, we plotted observed versus predicted values (Figure 3 C). We were able to see a very good correlation of the measured and the predicted response data for viable cell count. We also observed a very good reproducibility (0.99) of the center points indicating a low variability.

Figure 4



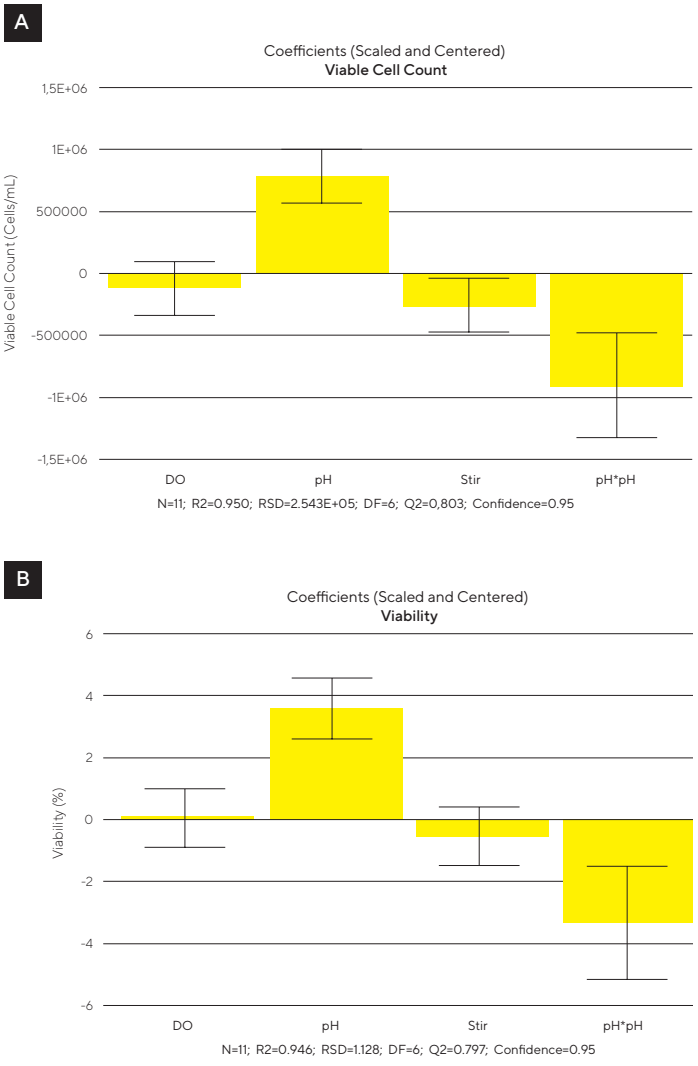
Note. A: Comparison of the viability of the cells grown in the Ambr® 15 Cell Culture under different cultivation conditions. Each line represents a different condition, as determined through the DOE. B: Response contour plot of the viability in relation to pH, DO and stirring speed. C: observed-versus-predicted plot.

When analyzing the viability, we also observed some differences, which depended on the chosen conditions of the vessels (Figure 4 A). However these differences were smaller than those for the viable cell count. According to the graph of the viability profile of all vessels, nearly all of them remained at a viability of >90 %.

Again, we can see in the contour plot, which process parameter combinations produced the best cell viability. (Figure 4 B). The highest viability values were obtained at a pH above 7.1. We identified that the model validity for the viability is very good (0.67), as well as the correlation of the observed and the predicted values (R^2=0.95) (Figure 4 C).

We also checked which process parameters had a significant impact on the responses, by analyzing the coefficients' plots (Figure 5).

Figure 5



Note. Coefficients plots of the readouts viable cell count (A) and viability (B) from the analysis of the DOE with MODDE®.

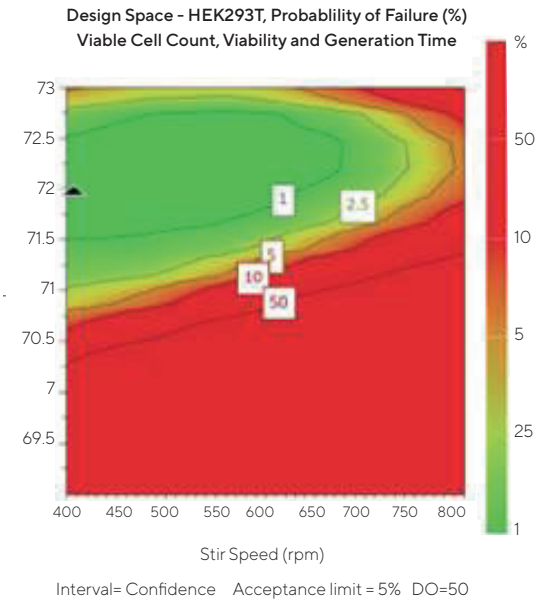
For the viable cell count, we found the pH and its quadratic term and stirring speed to be significant responses (Figure 5 A), since their mean value was higher than their variability. For viability, only pH and its quadratic term had a significant influence on the response (Figure 5 B). In this study, it was observed that pH positively correlates to both viability and viable cell density, meaning that with increased pH, the other two responses also increased. Since pH has a quadratic term, there is a certain optimum value, above which a further increase of the pH no longer leads to increased cell count and viability.

Stirring speed correlated negatively to the viable cell count: as stirring speed decreased, the viable cell count increased.

We also investigated the design space of our experimental setup (Figure 6).

Figure 6

Design Space and Optimal Process Parameter Setpoint from the Analysis of the DOE with MODDE®.



In Figure 6, low probability of failure values are colored in green and high values in red. Ideally, a final process should run within the setpoint range that has a low probability of failure. With the MODDE® software, we were able to identify an optimal setpoint with a low probability of failure, which was a stirring speed of 400 rpm, a pH of 7.2 and a percentage of DO of 50 % (Table 2), which was calculated with the Optimizer function. It is important to enter reasonable minimum, maximum, and target values of your responses, in order to model the design space. MODDE® can also predict the response values if the

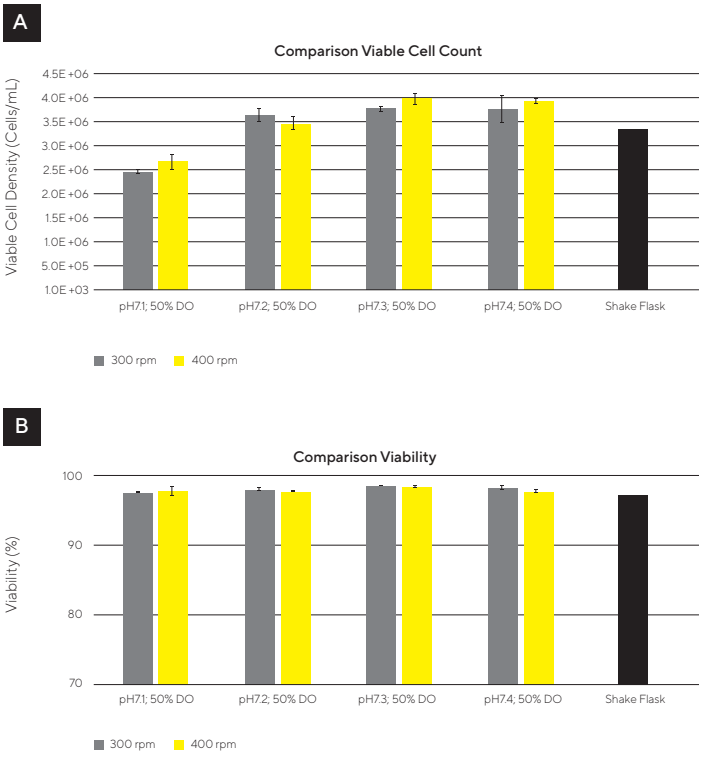
process is running at the optimal settings. For our process this would be a viable cell count of 2.9x10⁶ cells/mL and 98 % viability at the end of the cultivation.

The contour plot for viable cell count (Figure 3) and the design space (Figure 6), indicate that a stirring speed lower than 400 rpm could yield better cultivation results. To test this hypothesis, we performed a follow-up experiment. Since DO was not found to be a significant parameter we choose 50 % DO as a setpoint for further optimization studies.

Experiment 2

In the second experiment we wanted to confirm the results obtained in the first experiment. We also tested a lower stirring speed, namely 300 rpm, and 400 rpm again, as in the experiment before. Again we investigated the effect of the pH on cell growth by testing four different pH values, and performed two replicates of each condition. Additionally, we compared the cultivation in Ambr® 15 Cell Culture with a standard shake flask cultivation.

Figure 7



Note. Comparison of the viable cell count (A) and viability (B) of the cells cultured under different conditions with the Ambr® 15 and with a shake flask.

According to Figure 7 A the cultivation in the Ambr® 15 yielded up to 20 % increase in viable cell count, compared to a standard shake flask. Significant differences in cell growth were observed, depending on the chosen conditions of the vessel. Again, it was shown that the viable cell concentration was dependent on the culture pH value. Cells generally grew better at higher pH values like 7.3 and 7.4, rather than at lower pH values around 7.1. However, we did not observe a significant difference in cell growth between 300 and 400 rpm stirring speed.

The viability of the cells was generally better in Ambr® 15 than in a shake flask, but not depending on the pH or the stirring speed at least for this experimental setup (Figure 7 B).

An overall high level of reproducibility between replicate vessels was observed. A pH of 7.3 at 400 rpm stirring speed were determined as optimal for cell growth. This is in line with the optimal setpoints determined using Optimizer function of the MODDE® software from our first experiment.

Table 2

Response	Ambr® 15	Shake flask
VCC (cells/mL)	4.01x10⁶	3.35x10⁶
Viability (%)	98.4	97.5

Note. Overview of viable cell count and viability obtained after a five cultivation of HEK293T cells under optimized conditions in an Ambr® 15 and a shake flask.

At this optimal setpoint we were able to achieve a viable cell concentration of 4.01x10⁶ cells/mL in the Ambr® 15 system compared to 3.35x10⁶ cells/mL in the shake flask (Table 3).

Additionally, the viability of the cells in the Ambr® 15 was 98.4 % and thereby higher than in the shake flask, in which the cells had a viability of 97.5 %.

Conclusion

This study demonstrates that the Ambr® 15 microbioreactor system in combination with the DOE software MODDE® enables a systematic investigation of critical process parameters and rapid, high throughput process development and optimization.

The results prove the following:

- The main benefits of making the transition from using shake flasks to Ambr® 15 are:
  - capacity to screen many conditions in parallel
  - control of process parameters including pH and DO
- The Ambr® 15 allows for the selection of optimal culture process parameters or clones, in a high-throughput manner, while mimicking the large scale stirred tank bioreactors such as Biostat STR® [7]. The system alleviates the need to adapt from shake flasks to stirred systems, and facilitates the transfer of well characterized and optimized processes to larger scales.
- The Ambr® 15 Cell Culture | MODDE® combination, performed as an ideal system for parallel assessment of various cultivation parameters, exemplified in the optimized cultivation of HEK293T cells. This paves the way for using the system in cellular immunotherapy and gene therapy bioprocess development and optimization.

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References

[1] Aziza Manceur, Howard Kim, Vanja Misic, Nadejda Andreev, July Dorion-Thibaudeau, Stéphane Lanthier, Alice Bernier, Sonia Tremblay, Anne-Marie Gelinas, Sophie Broussau, Rénald Gilbert, Sven Ansorge (2017). Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. Human Gene Therapy Methods. 28. 10.1089/hgtb.2017.086.

[2] Yu Hua Chen, Celeste Pallant, Christopher J. Sampson, Martijn H. Brugman, Steven J. Howe, Conrad A. Vink (2020). Rapid Lentiviral Vector Producer Cell Line Generation Using a Single DNA Construct. Molecular Therapy: Methods & Clinical Development, volume 19, P47-57

[3] Eugenio Macchiarulo, Phil Bassett, Mark Dudley (2018). Challenges and progress in lentiviral vector bioprocessing. Cell Gene Therapy Insights 4(10), 915-925. 10.18609/cgti.2018.091

[4] Vijay Janakiraman, Chris Kwiatkowski, Rashmi Kshirsagar, Thomas Ryll, Yao-Ming Huang (2015). Application of high-throughput mini-bioreactor system for systematic scale-down modeling, process characterization, and control strategy development. Biotechnology Progress, Volume 31, Issue 6.

[5] The Benefits of High Throughput Process Development. CPI.com

[6] Franziska Bollmann (2020). Optimized Suspension-Based Production of Lentiviral Vectors with a DOE Approach. Tutorial, Genetic Engineering & Biotechnology News.

[7] Sebastian Ruhl, Naomi de Almeida, Melisa Carpio, Jens Rupprecht, Gerhard Greller, Jens-Christoph Matuszczyk (2020). A Rapid, Low-Risk Approach Process Transfer of Biologics from Development to Manufacturing Scale; BioProcess International 18(5).

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