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

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Introduction

- HEK293T cells are commonly used as a workhorse cell line for viral vector production for cell and gene therapy applications.
- A significant challenge for the Regenerative Medicine (RM) industry is to develop a HEK293T suspension cell culture process that is well characterized and can be scaled up for production to ensure clinical and commercial success.
- Ambr® 15 Cell Culture is an automated micro-scale bioreactor system that mimics the features and process control (pH, DO, temperature, stirring rate) provided by much larger scale bioreactors, but in a volume of 10 - 15 mL. Parallel processing capability and excellent consistency enable rapid, high throughput process improvement and optimization, including DoE studies.
- High throughput tools with parallel processing, such as Ambr® 15, help to address a major manufacturing bottleneck. They can be used as a scale-down model for process development, clone selection and effective media optimization in less time with reduced reagent use and labor saving.
- Design of Experiments (DoE) is a rational and cost-effective approach to practical experimentation that allows the effect of variables to be assessed using only the minimum of resources. MODDE® is a state-of-the-art DoE software package. It enables fast and effective identification of critical process parameters (CPPs) and, subsequently, establishment of a Design Space, resulting in reduced bioprocess complexity and increased process understanding.

Scope of Work

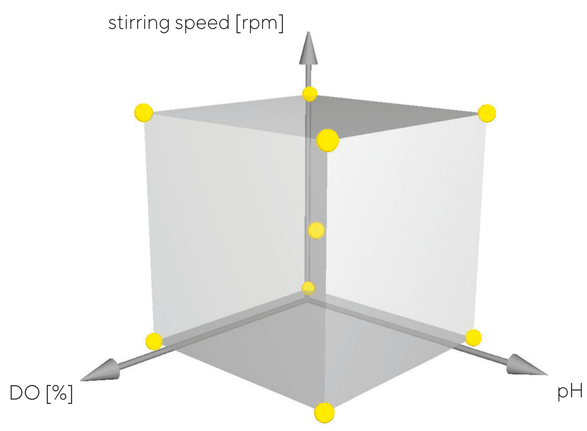
- Use Ambr® 15 for HEK293T suspension culture optimization aiming for VCC (viable cell count/density), viability and generation time to be comparable to standard shake flask culture: identify optimum stirring speed, DO value and pH value.
- Perform a DoE study to identify optimal culture conditions by using MODDE® software for experimental planning and analysis of results.

Materials and Methods

- Adherent HEK293T cells have been adapted to suspension culture in serum-free conditions
- optimal culture medium was CD293 + 4mM Glutamax (Gibco) as determined in a previous study
- Setpoints Ambr® 15 culture using sparged vessels:
  - bioreactor temperature: 36.8°C
  - inoculation density: 3x105 cells/mL
  - fill volume: 15 mL, inoculum volume: 2 mL
  - daily antifoam c addition
- with MODDE® software a 2-level full factorial design with three centerpoints was used for setting up a DoE (experiment 1)
- setpoints shake flask:
  - incubator temperature: 36.8°C
  - inoculation density: 3x105 cells/mL
  - shaking rate: 120rpm (baffled flask)
  - fill volume: 37.5mL
  - CO^2: 8%
  - orbit: 5cm

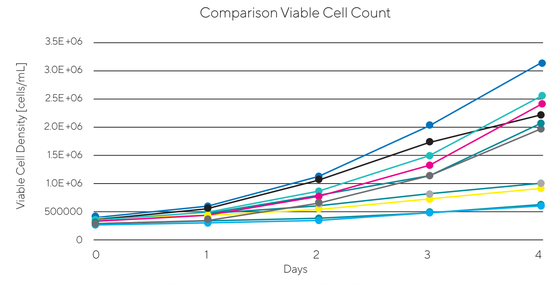
Table 1: overview of process parameters and readouts of the DoE study

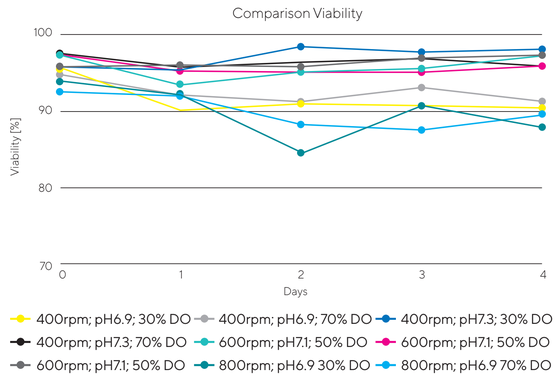
Process Parameters	Range	
Stirring speed [rpm]	400	800
pH	6.9	7.3
DO [%]	30	70
Responses	VCC, viability, generation time	

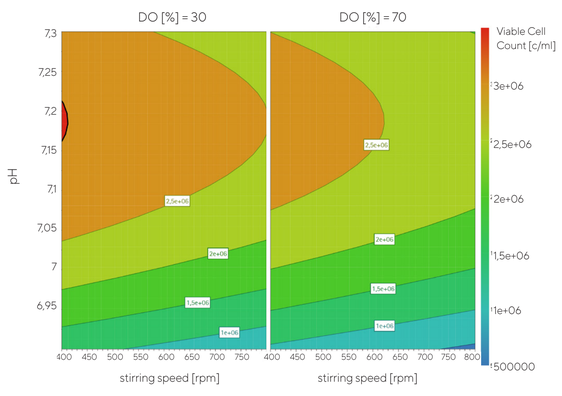


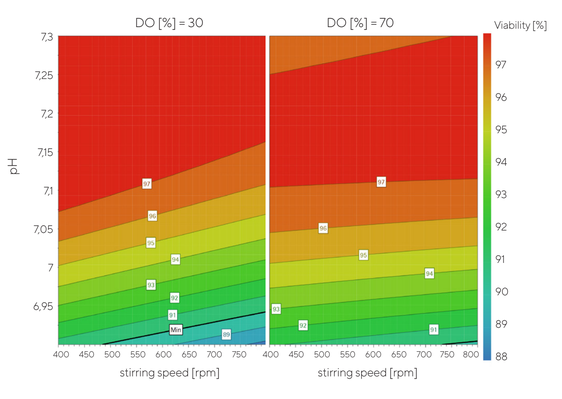
Results: Experiment 1

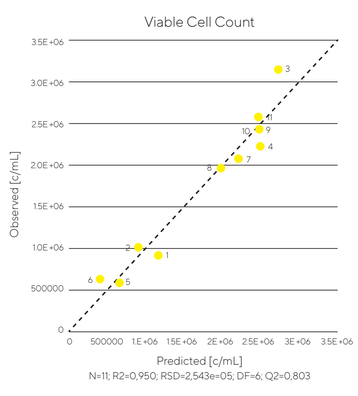
DoE study for optimization of HEK293T cultivation

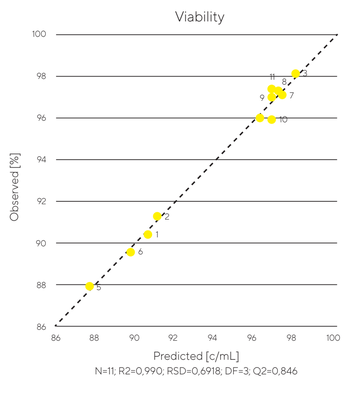


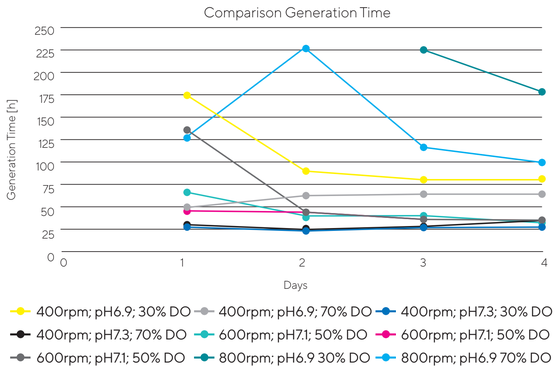


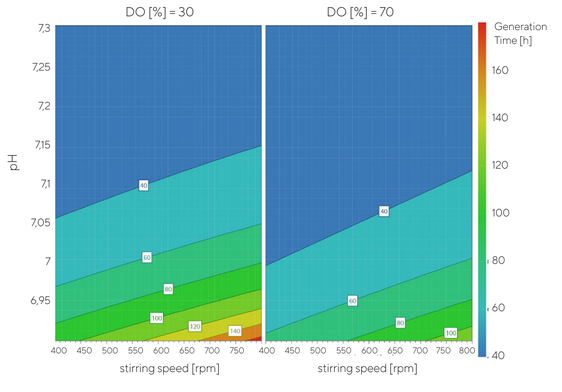


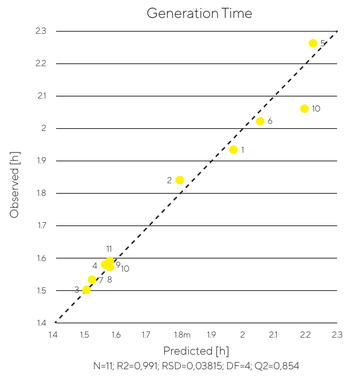










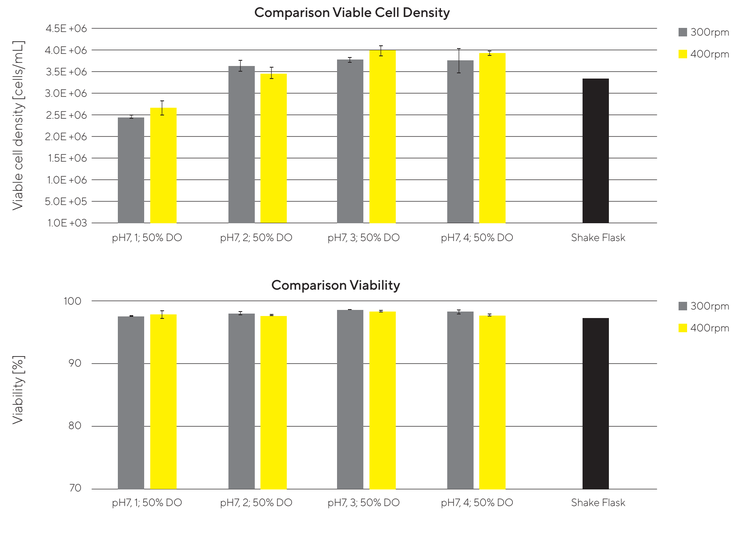


Results: DoE follow up Experiment 2

Comparison of Ambr® 15 with shake flask culture

selected conditions: analyzed in duplicates

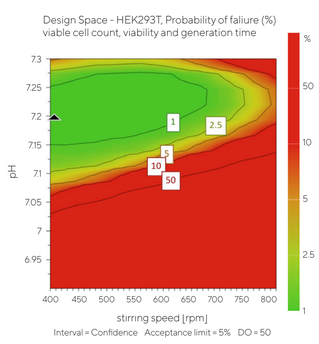
- 2 stirring speeds: 300 and 400 rpm and 4 pH values: 7.1, 7.2, 7.3, 7.4
- cultivation in the Ambr® 15 yields increased viable cell count compared to standard shake flask
- Ambr® 15 performs equal to shake flask wrt generation time
- viability of cells is generally better in Ambr® 15 than in shake flask
- overall high level of reproducibility between replicate vessels observed
- pH 7.3 at 400 rpm stirring speed determined to be optimal for cell growth
- generation time and VCC are dependent on pH value and stirring speed



Response	Ambr® 15	Shake flask
VCC [cell/mL]	$4.01x10^6$	$3.35x10^6$
Viability [%]	98.4	97.5
Generation time [h]	28.8	28.1

Results: Find optimal settings within design space to fulfill acceptance criteria:

- DoE evaluation in MODDE® showed a good model validity
- pH and stirring speed were identified to be significant parameters
- optimal setpoint: stirring speed 400 rpm; pH 7.22; DO=30% according to Optimizer function in MODDE®
- according to contour plot: possibility that stirring speed <400 rpm could yield better cultivation results
- since DO was not found to be a significant parameter we choose 50% DO as a setpoint for further optimization studies



Key process attributes	Unit	Optimization Objective	Min	Target	Max
Viable cell count	c/ml	Maximize	2.00E+06	3.00E+06	-
Viability	%	Maximize	95	100	-
Generation time	h	Maximize	-	20	40

Prob. of faliure	Predicted output at optimum
0.02%	2.9E+06
0.17%	98.0
0.29%	29.9

Process parameter	Unit	Role	Value	Low limit	High limit
DO	%	Constant	50	-	-
pH		Free	-	6.9	7.3
Stirring speed	rpm	Free	-	400	800

Optimal settings
50
7.2
400

Summary and Outlook

This study demonstrates that the Ambr® 15 Cell Culture system in combination with the DoE MODDE® software 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 very fast. A key next step is to use the identified HEK293T culture conditions to perform a DoE study with the Ambr® 15 to optimize viral vector production for cell and gene therapy applications.

Process parameters	Optimized cultivation setpoints Ambr® 15
Stirring speed [rpm]	400
pH	7.3
DO [%]	50