Simplifying Progress

Optimizing T Cell Expansion in Ambr® 15 Cell Culture Using DoE and Process Transfer to Ambr® 250 Modular

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Aims

Manufacturing remains challenging for many therapeutic cellular products, including CAR-T cells. A critical step in the manufacturing process is T cell expansion as it ensures that the required cell number and cell quality is obtained. Establishing a scalable process in stirred culture systems offers many advantages over static culture, enabling effects of agitation and other process control variables to be investigated earlier on in the process development lifecycle.

In this case study, we establish and optimize T cell culture in stirred tank bioreactors using a DoE approach. We aim to show that the Ambr® platform in combination with the MODDE® software accelerates process development for cell-based therapies and provides means for better process understanding.

Study Overview

The study was set up in three main steps as described below and shown in Figure 1:

Step 1: Establishment of T cell culture under agitated conditions in the Ambr® 15 Cell Culture system and screening of various T cell media under agitated and static conditions

Step 2: Applying a DoE approach using the MODDE® software, process parameters (pH, DO, stirring speed, IL-2 concentration, seeding density) were investigated in Ambr® 15 Cell Culture to determine optimum conditions

Step 3: Based on results from Step 2, T cell expansion was transferred to larger scale bioreactors in Ambr® 250 Modular

Figure 1. Study overview. An Ambr® 15 Cell Culture 24 way system with spargless vessels was used for this study

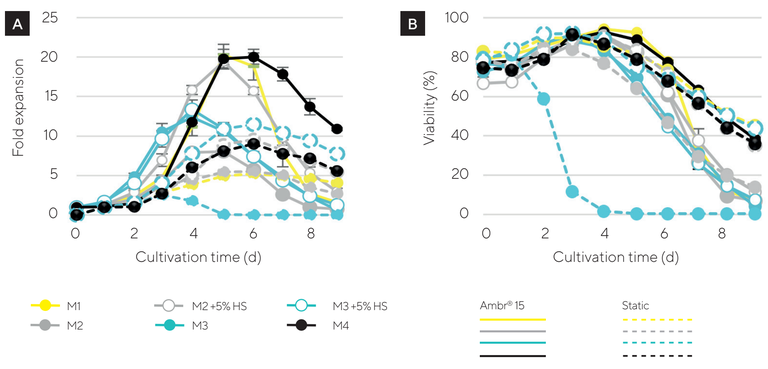
Step 1: Screening of Commercially Available Media

- Screening of 4 different commercially available media (M1 - M4)
- Two media, recommended to be used with serum (media 2 and 3), were tested with and without serum supplementation
- Comparison of static and stirred culture conditions showed differences in media performance
- Overall a higher fold expansion was achieved in the stirred cultures, compared to static cultures
- Medium 3 + human serum (HS) showed the highest fold expansion under static conditions but did not perform as well in stirred culture
- Media 1 and 4 were chosen for step 2: serum-free and highest fold expansion in Ambr® 15 (stirred conditions)

Table 1: Ambr® 15 Cell Culture parameters for media screening

Parameter	
Stirring speed [rpm]	300
DO [%]	60
pH	7.2
Seeding density [cells/mL]	5×10^5 (in 10 mL)
Feed	Fed batch (day 2 to 15 mL)
IL-2 [U/mL]	400
Cells	Activated primary human T cells from cryopreserved hPBMC

Figure 2: Screening of media. Pre-cultured T cells were expanded in Ambr® 15 (n=3 replicates) or static well plates and T flasks (n=1) in a humidified 5% CO_2 incubator. Inoculum for agitated and static cultures was derived from the same cell pool and expanded under the respective condition using the same medium batches and feeding on day 2. Fold expansion (A) and viability (B) was determined



Step 2: Optimize T Cell Expansion in Ambr® 15 Cell Culture Using DoE

First Optimization:

- Batch culture in 10 mL culture volume using pre-cultured, activated T cells
- Using MODDE® software a linear, reduced combinatorial design with replicates of 4 vessels was chosen (24 experiments in total; all available vessels on Ambr® 15 Cell Culture 24 vessel system were filled)
- Factors for evaluation: see Table 2
- Response: fold expansion and viability on day 3

- Good model for fold expansion:
  - Good model fit (high R^2), high predictive power and model validity (data not shown)
  - Positive impact: IL-2, pH and medium 1
  - Negative impact: high seeding density and medium 4 (Figure 3A) ;
  - Contour plot (Fig. 3C): highest fold expansion at low seeding density and high IL-2 concentration
- Poor model for viability:
  - Low R^2, predictive power and model validity (data not shown) -> model should not be used for predictions
  - Indication of positive IL-2 effect (Fig. 3B)
- Continue with low seeding density, medium 1, 400 rpm and narrow pH range (7.2 – 7.4) for second optimization

Table 2: Factors screened for T cell expansion

Parameter	
Stirring speed [rpm]	300; 400
DO [%]	50; 70; 90
pH	7.0; 7.3; 7.5
Medium	M1; M4
Seeding density [cells/mL]	5×10^5; 1×10^6
IL-2 [U/mL]	50; 125; 200

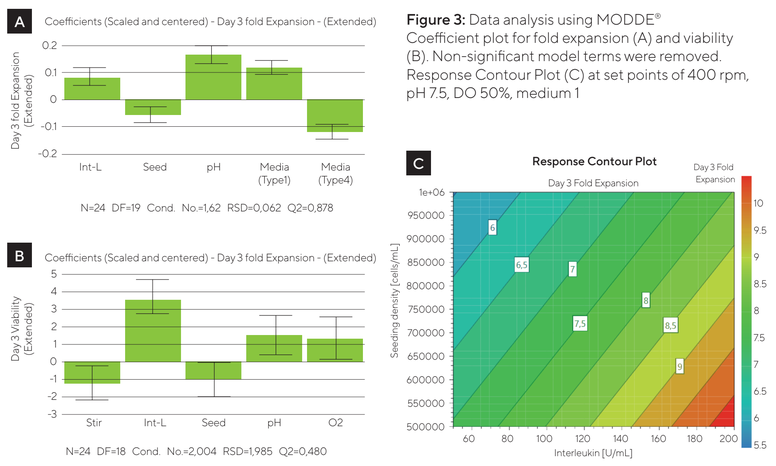


Figure 3: Data analysis using MODDE® Coefficient plot for fold expansion (A) and viability (B). Non-significant model terms were removed. Response Contour Plot (C) at set points of 400 rpm, pH 7.5, DO 50%, medium 1

Second Optimization:

- Reduced number of factors (Table 3)
- Two responses: fold expansion and viability on day 3
- Quadratic reduced combinatorial design with 6 replicate vessels was chosen (24 experiments)

- Good model for fold expansion and viability:
  - Coefficient plots (Fig. 4A and 4B):
    - IL-2 most important factor for fold expansion and viability
    - Indication that higher DO may have a negative effect
    - Weak effect of pH
- Sweet Spot Plot (Fig. 4C) illustrate the optimal area (in green) where both fold expansion and viability are high:
  - Optimal area at settings of IL-2 > 164 U/mL and low DO
  - As IL-2 concentrations increase above 164 U/mL, the DO range can be increased and the sweet spot will still be met
  - IL-2 was identified as a critical factor with high impact on cell growth and viability
  - pH was identified as a critical factor during screening of wide pH range; high pH impact reduced by narrowing the pH range

Table 3: Reduced number of factors for second DoE optimization. Other factors were kept constant (Medium 1, 400 rpm, seeding density: 5×10^5 cells/mL, culture volume: 10 mL, batch culture

Factors	Second optimization
DO [%]	50; 70; 90
pH	7.2; 7.4
IL-2 [U/mL]	50; 125; 200

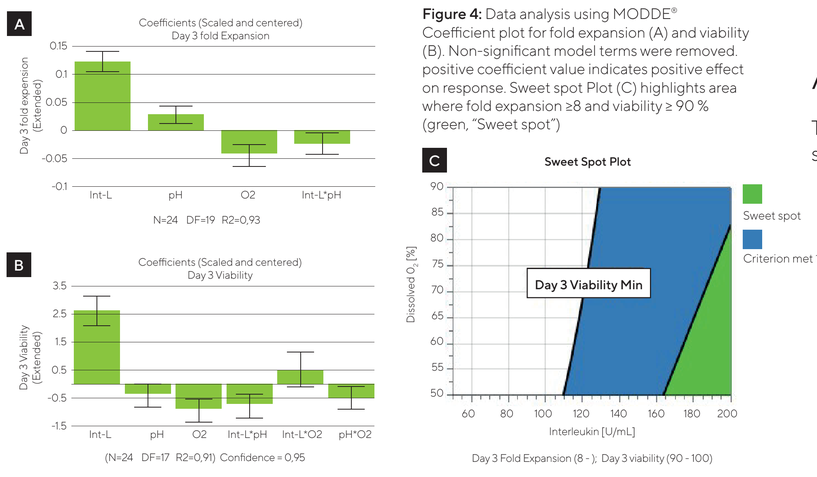


Figure 4: Data analysis using MODDE® Coefficient plot for fold expansion (A) and viability (B). Non-significant model terms were removed. positive coefficient value indicates positive effect on response. Sweet spot Plot (C) highlights area where fold expansion ≥8 and viability ≥ 90 % (green, "Sweet spot")

Step 3: Process Transfer to Ambr® 250 Modular

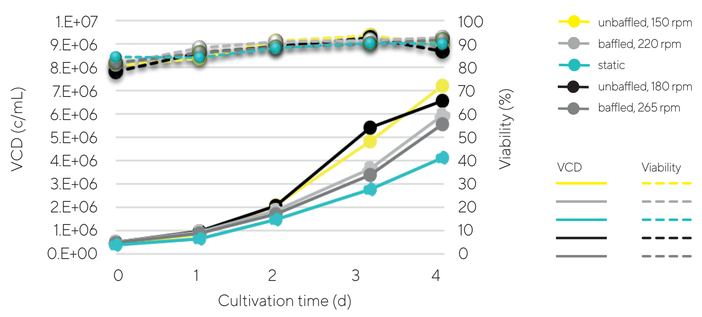
- Process transfer to larger scale bioreactor system: Ambr® 250 Modular (Fig. 5)
- Baffled and unbaffled vessels were tested
- Process parameters are shown in Table 4
- Control culture was expanded using the same medium and cells in static T flask in a humidified 5% CO_2 incubator
- Higher cell growth in both Ambr® 250 vessel types than in static control (Fig. 6)
- Cell growth in baffled vessel slightly higher than in unbaffled vessels (Fig. 6)
- Viability comparable for all cultures (Fig. 6)

Table 4: Parameters for Ambr® 250 Modular experiments: Two baffled and two unbaffled bioreactor vessels were tested in parallel with high and low stirring speeds. For comparison of the two different vessels, P/V was kept constant and stirring speeds were adapted accordingly

Parameter	
DO [%]	50
IL-2 [U/mL]	200
pH	7.2
Medium	M1
Feed	Batch
Seeding density [c/mL]	5×10^5
Cells	Pre-cultured and activated; from cryopreserved hPBMC
Vessel type	Baffled | unbaffled
Stirring speed [rpm]	220; 265 | 150; 180

Figure 5: Ambr® 250 Modular Benchtop system with 3 modules. Each module holds two ready to use bioreactor vessels with attached reagent reservoirs

Figure 6: Cell expansion in Ambr® 250 Modular. Cell expansion was performed in baffled and unbaffled bioreactor vessels using two stirring speeds (cacluated based on constant P/V for both vessel types. A static control culture (T flask) was performed in parallel. Solid lines = viable cell density (VCD), dotted lines = viability



Conclusions

Ambr® 15 Cell Culture can be used in a time- and cost-efficient manner to establish T cell culture under stirred conditions and to screen various T cell media. In both the Ambr® 15 and Ambr® 250 systems higher cell growth was achieved in the stirred bioreactors when compared to static cultures. Using a DoE approach, the Ambr® 15 Cell Culture platform in combination with the MODDE® software allows for a rapid and systematic evaluation of critical process parameters (CPP) to optimize T cell expansion.

Acknowledgements

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